

FORM PTO-1390
(REV. 1-98)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

55411.000002

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/242657

INTERNATIONAL APPLICATION NO.

PCT/DK97/00342

INTERNATIONAL FILING DATE

August 25, 1997

PRIORITY DATE CLAIMED

August 23, 1996

TITLE OF INVENTION

ARTIFICIAL PROMOTER LIBRARIES FOR SELECTED ORGANISMS AND
PROMOTERS DERIVED FROM SUCH LIBRARIES

APPLICANT(S) FOR DO/EO/US

Peter Ruhdal JENSEN and Karin HAMMER

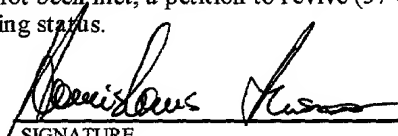
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

SEE ATTACHMENT 1 ON PAGE 1A

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
		PCT/DK97/00342		55411.000002	
17. <input type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1070.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$840.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$790.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$720.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$98.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
				\$ 840.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	22 - 20 =	2	x \$18.00	\$ 36.00	
Independent claims	2 - 3 =	0	x 0	\$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 876.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$ 0	
SUBTOTAL =				\$ 876.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0	
TOTAL NATIONAL FEE =				\$ 876.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$ 0	
TOTAL FEES ENCLOSED =				\$ 876.00	
				Amount to be refunded:	\$
				charged:	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>876.00</u> to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>50-0206</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
Stanislaus Aksman, Esq. Hunton & Williams 1900 K Street, NW, Suite 1200 Washington, DC 20006 Phone: (202) 955-1500 Facsimile: (202) 778-2201			 SIGNATURE		
			<u>Stanislaus Aksman</u> NAME		
			<u>28,562</u> REGISTRATION NUMBER		

ATTACHMENT 1

1. Notice Informing the Applicant of the Communication of the International Application to the Designated Offices (Form PCT/IB/308)
2. Published PCT application with International Search Report
3. Notification Concerning Submission of Priority Documents
4. First Written Opinion of June 29, 1998 and response of August 26, 1998
5. Second Written Opinion of November 19, 1998 and response of November 26, 1998
6. International Preliminary Examination Report, including copies of amendments under Article 34 comprising: amended specification sheet 10a and new claims 1-22
7. Letter of January 14, 1999 from H. Sogaard to International Preliminary Examining Authority and January 15, 1999 Notification Transmittal of the International Preliminary Examination Report, sending to H. Sogaard (of Plougmann, Vingtoft & Partners) the corrected sheet 2 of the IPER dated December 10, 1998
8. Sequence Listing, including (i) hard copy (pages 38-69P); (ii) diskette; (iii) Statement Under 37 CFR Section 1.821
9. Request to Examine Application on the Basis of the Text Amended Under Article 34 in the PCT

300 Rec'd PCT/US 19 FEB 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of :)	
)	
Peter Ruhdal JENSEN et al.)	Group Art Unit: To Be Assigned
)	
Serial No.: To be Assigned)	Examiner: To Be Assigned
)	
Filed: Herewith)	

For: ARTIFICIAL PROMOTER LIBRARIES FOR SELECTED ORGANISMS
AND PROMOTERS DERIVED FROM SUCH LIBRARIES

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to the examination of the above-identified patent application, please amend
the application as follows:

IN THE CLAIMS

Claim 7, line 1, delete "or 6".

Claim 9, line 1, change "any of claims 5-8" to - - claim 5 - -.

Claim 14, line 1, delete "or 13".

Claim 15, line 1, change "any of claims 12-14" to - - claim 12 - -.

17. (Amended) A method according to claim 16 wherein the set of different
promoters obtained is a promoter library [according to any of claims 1-15] spanning,
with respect to promoter activities for said gene, a range of interest, in small steps, each
step preferably changing the activity by 50-100%.

Claim 18, line 3, change "any of claims 1-15" to -- claim 1 --.

Claim 21, line 3, delete "or 17".

REMARKS

I. SOME CLAIMS ARE AMENDED TO ELIMINATE MULTIPLE DEPENDENCY

Claims 7, 9, 14, 15, 17, 18 and 21 are amended to eliminate multiple dependency.

II. REQUEST FOR ALLOWANCE

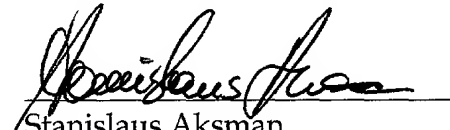
An indication of allowance of all claims is solicited.

Respectfully submitted,

HUNTON & WILLIAMS

Date: February 19, 1999

By:


Stanislaus Aksman
Registration No. 28,562

1900 K Street, N.W., Suite 1200
Washington, D.C. 20006-1109
Telephone: (202) 955-1500
Facsimile: (202) 778-2201

09/242657

Attorney Docket No. 55411.000002

19 FEB 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of :)
Peter Ruhdal JENSEN et al.) Group Art Unit: To Be Assigned
Serial No.: To be Assigned) Examiner: To Be Assigned
Filed: Herewith)

For: ARTIFICIAL PROMOTER LIBRARIES FOR SELECTED ORGANISMS
AND PROMOTERS DERIVED FROM SUCH LIBRARIES

**SUBMISSION OF SEQUENCE LISTING AND
STATEMENT UNDER 37 C.F.R. § 1.821**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with the provisions of 37 C.F.R. § 1.821, Applicants submit herewith a paper copy of "Sequence Listing", pages 38-69P, which is substantially the same as the Sequence Listing included on pages 38-69 of the Published International Application, WO 98/07846. To maintain pagination consistent with pages 38-69 of the Published International Application, Applicants numbered the attached paper copy of Sequence Listing as pages 38-69 and 69A-69P. On the attached paper copy of "Sequence Listing" Applicants added the following formal items which were not included on pages 38-69 of the Published International Application:

- identification of the second inventor, Karin Hammer
- identification of the Correspondence Address
- current Application Data

- identification of the PCT Application.

Aside from those additions, the enclosed paper copy of Sequence Listing is the same as the Sequence Listing on pages 38-69 of the Published International Application.

Applicants request entry of the "Sequence Listing" into the record.

Applicants also submit herewith a computer readable copy of the "Sequence Listing" on a 3½ inch diskette, in ASCII format as required by 37 C.F.R. § 1.821(e).

The undersigned attorney, Stanislaus Aksman, hereby states that the content of the computer readable disk copy of the "Sequence Listing" and the paper copy of the "Sequence Listing" submitted herewith are the same. No new matter is introduced by the "Sequence Listing".

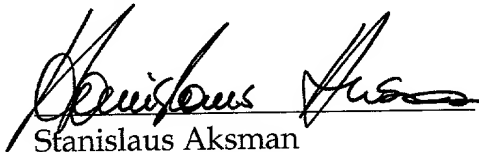
To the extent necessary, the Commissioner is authorized to grant any extension of time deemed needed for entry of this document and to charge additional fees associated with this communication or such extension of time or credit any overpayment to Deposit Account No. 50-0206. A duplicate copy of this communication is enclosed for accounting purposes.

Respectfully submitted,

HUNTON & WILLIAMS

Date: February 19, 1999

By:


Stanislaus Aksman
Registration No. 28,562

1900 K Street, N.W., Suite 1200
Washington, D.C. 20006-1109
Telephone: (202) 955-1500
Facsimile: (202) 778-2201

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS:

(A) NAME: Peter Ruhdal Jensen
(B) STREET: Soegaardsvej 19
(C) CITY: Gentofte
(E) COUNTRY: Denmark
(F) POSTAL CODE (ZIP): DK-2820

(A) NAME: Karin Hammer
(B) STREET: Gaerdesmuttevej 20
(C) CITY: Hoersholm
(D) COUNTRY: Denmark
(E) POSTAL CODE (ZIP): DK-2970

(ii) TITLE OF INVENTION: Artificial promoter libraries for selected organisms and promoters derived from such libraries

(iii) NUMBER OF SEQUENCES: 58

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CORRESPONDENCE ADDRESS:

Stanislaus Aksman
Hunton & Williams
1900 K Street, NW
Washington, DC 20006-1109
Phone: (202) 955-1500
Facsimile: (202) 778-2201

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE IN THE US PTO: February 19, 1999
(C) ATTORNEY DOCKET NO.: 55411.000002

666727/394666

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: DK 886/96
- (B) FILING DATE: 23-AUG-1996
- (C) PCT APPLICATION NUMBER: PCT/DK97/00342
- (D) FILING DATE: August 25, 1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:26..82
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Artificial promoter library"
/note= "A degenerated sequence specifying a mixture of
artificial promoters covering a wide range of expression in
small steps in *L. lactis*"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:31..45
- (D) OTHER INFORMATION:/standard_name= "Consensus
sequence"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:60..69
- (D) OTHER INFORMATION:/standard_name= "Consensus
sequence"

(ix) FEATURE:

- (A) NAME/KEY: -35_signal
(B) LOCATION:40..45
(D) OTHER INFORMATION:/standard name="-35 box"

(1X) FEATURE:

- (ix) **FEATURE:**

- (A) NAME/KEY: misc_recomb
(B) LOCATION:74..98
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Multiple cloning site"
/label= MCS
/note= "A sequence specifying recognition sites for the
restriction endonucleases: ScaI, RsaI, HpaI, HincII, MseI, SfcI,
PstI, Fnu4HI, BbvI, PvuII, NspBII, AluI, EcoRI."

CGGGATCCTT AAGAATATTA TGCATNNNNN AGTTTATTCT TGACANNNNN
NNNNNNNNNT 60

GGTATAATAN NANAGTACTG TTAAGTGCAG CTGAATTCGG
100

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 113 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:23..95
- (D) OTHER INFORMATION:/standard_name= "Artificial promoter library"
/note= "A degenerated sequence specifying a mixture of artificial temperature regulated promoters covering a wide range of expression in small steps in *L. lactis*"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:23..49
- (D) OTHER INFORMATION:/standard_name= "Sequence providing temperature regulation to promoters"
/note= "This sequence comprising two inverted repeats separated by a short spacer provides temperature (heat shock) regulation to promoters in Gram-positive bacteria"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:50..60
- (D) OTHER INFORMATION:/standard_name= "Consensus sequence"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:75..84

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:89..95
(D) OTHER INFORMATION:/standard_name= "Consensus
sequence"

(ix) FEATURE:
 (A) NAME/KEY: -10_signal
 (B) LOCATION:78..83
 (D) OTHER INFORMATION:/standard name= "Pribnow box"

(ix) FEATURE:
 (A) NAME/KEY: misc_recomb
 (B) LOCATION:89..111
 (D) OTHER INFORMATION:/standard_name= "Multiple cloning
 site"
 /label= MCS
 /note= "A sequence specifying recognition sites for the
 restriction endonucleases: ScaI, RsaI, SfcI, PstI, Fnu4HI, BbvI,
 PvuII, NspBII, AluI, XbaI, MaeI, EcoRI, ApoI."

CGGGATCCAA GCTTAATATT AATTAGCACT C>NNNNNNNNN GAGTGCTAAT
TTTTTTGACA 60

NNNNNNNNNN NNNNTGGTAT AATANNANAG TACTGCAGCT GTCTAGAATT
CGG 113

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 199 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: protein_bind
- (B) LOCATION:10..16
- (D) OTHER INFORMATION:/function= "Activating promoters in *S. cerevisiae*"
/bound_moiety= "GCN4 protein"
/standard_name= "Upstream activating sequence"
/label= UAS_GC4p
/note= "A DNA sequence that specifies a binding site for the GCN4 protein, which activates the transcription of genes involved in aminoacid synthesis in *S. cerevisiae*."

(ix) FEATURE:

- (A) NAME/KEY: TATA_signal
- (B) LOCATION:67..72
- (D) OTHER INFORMATION:/standard_name= "TATA box"

(ix) FEATURE:

- (A) NAME/KEY: misc_signal
- (B) LOCATION:122..144
- (D) OTHER INFORMATION:/function= "Transcription initiation"
/standard_name= "TI box"

(ix) FEATURE:

bioRxiv preprint doi: <https://doi.org/10.1101/201709>; this version posted September 11, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

- (A) NAME/KEY: protein_bind
- (B) LOCATION:122..144
- (D) OTHER INFORMATION:/bound_moiety= "Arginine repressor"
/standard_name= "arginine repressor binding site"
/label= argR

(ix) FEATURE:

- (A) NAME/KEY: misc_RNA
- (B) LOCATION:145..192
- (D) OTHER INFORMATION:/function= "Spacer"
/standard_name= "Part of native sequence for ARG8
gene incl. first codon"

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION:3..8
- (D) OTHER INFORMATION:/standard_name= "Recognition site
for restriction endonuclease EcoRI"
/label= EcoRI_site

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION:192..197
- (D) OTHER INFORMATION:/standard_name= "Recognition site
for restriction endonuclease BamHI"
/label= BamHI_site

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:10..192
- (D) OTHER INFORMATION:/standard_name= "Artificial
promoter library"
/note= "A degenerated sequence specifying a mixture of
artificial promoters covering a wide range of expression in
small steps in S. cerevisiae"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAGAATTCGT GACTCANNNN NNNNNNNNNN NNNNNNNNNN
NNNNNNNNNN NNNNNNNNNN 60

NNNNNNNNNN NNNNNNTATA AANNNNNNNN NNNNNNNNNN
NNNNNNNNNN NNNNNNNNNN 120

NCTCTTAAGT GCAAGTGACT GCGAACATTT TTTTCGTTTG TTAGAATAAT
TCAAGAATCG 180

CTACCAATCA TGGATCCCG

199

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas putida*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 1..45
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Artificial promoter library"
/note= "A degenerated sequence specifying a mixture of
artificial promoters covering a wide range of expression in small
steps *Pseudomonas putida*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

NNNNNNNNTT GRNNNNNNNNN NNNNNNNNNN NTATRATNNN NNNNN
45

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CATACCGGAG TTTATTCTTG ACAGTTCCAC CTCGGGTTGA TATAATATCT
CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional

promoter"
/label= Cp10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CATGGCTTAG TTTATTCTTG ACAGGGTAGT ATCACTGTGA TATAATAGGA
CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional

promoter"

label= Cp11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CATAAGTGAG TTTATTCTTG ACCCGGACGC CCCCTTTGA TATAATAAGT
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(B) LOCATION:4..60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CATATACAAG TTTATTCTTG AACTAGTCG GCCAAAATGA TATAATACCT
GAGTACTGTT 60

(i) SEQUENCE CHARACTERISTICS:

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CATGCTTTAC TTTATTCTTG ACAAACCAC CAGCTTTTGG TATAATACGT
GAGAACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CATGACGGAG TTTATTCTTG ACACAGGTAT GGACTTATGA TATAATAAAA
CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CATTACNTAG TTNATTCTTG ACAGAATTAC GATTCGCTGG TATAATATAT
CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..58

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp16

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CATTGTGTAG TTTATTCTTG ACAGCTATGA GTCAATTTGG TATAATAACA
GTACTCAG 58

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CATTCTGGAG TTTATTCTTG ACCGCTCAGT ATGCAGTGGT ATAATAGTAC
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..58
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATTTTGCAG TTTATTCTTG ACATTGTGTG CTCGGGTGT ATAATACTAA
GTACTGTT 58

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
(B) LOCATION:4..58
(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CATCGCTTAG TTTTCTTGA CAGGAGGGAT CCGGGTTGAT ATAATAGTTA
GTACTGTT 58

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) **HYPOTHETICAL: YES**

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
(B) LOCATION:4..60
(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CATTGCTAG TTTATTCTTG ACATGAAGCG TGCCTAATGG TATATTACTT
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CATGGGTGAG TTTATTCTTG ACAGTGCGGC CNGGGGCTGA TATCATAGCA
GAGTACTATT 60

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CATTACCGAG TTTATTCTTG ACACCGTTTA TCGGGGTTGT ATAATACTAT
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CATGTAGGAG TTTATTCTTG ACAGATTAGT TAGGGGGTGG TATAATATCT
CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CATGGGTAAG TTTATTCTTC AACTATCTG GGCCCGATGG TATAATAAGT
GACTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:3..59

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTTTGGCAGT TTATTCTTGA CATGTAGTGA GGGGGCTGGT ATAATCACAT
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp26

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CATTCTACAG TTTATTCTTG ACATTGCACT GTCCCCCTGG TATAATAACT
ATACATGCAT 60

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CATGGGGCCG TTTATTCTTG ACAACGGCGA GCAGACCTGG TATAATAATA
TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp29

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CATCGGTAAG TTATTCTTGA CATCTCAGGG GGGACGTGGT ATAATAACTG
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CATCCTGTAG TTTATTCTTG ACACACGTNN TTAGCTGTGG TATAATAGGA
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CATGACAGAG TTTATTCTTG ACAGTATTGG GTTACTTTGG TATAATAGTT
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CATACGGGAG TTTATTCTTG ACATATTGCC GGTGTGTTGG TATAATAACT
TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"

/label= Cp33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CATGTTGGAG TTTATTCTTG ACATACAATT ACTGCAGTGA TATAATAGGT
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp34

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CATCGCGAAG TTTATTCTTC ACACACCGCA GAACTTGTGG TATAATACAA
CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp37

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CATCATTAAG TTTATTCTTC ACATTGGCCG GAATTGTTGT ATAATACCTT
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CATAGAGAAG TTTATTCTTG ACAGCTAACT TGGCCTTTGA TATAATACAT
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CATTGCGAAG TTTATTCTTG ACAGTACGTT TTTACCATGA TATAATAGTA
TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GATGTTTTAG TTTATTCTTG ACACCGTATC GTGCGCGTGA TATAATCGGG
ATCCTTAAGA 60

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CATAGAACAG TTTATTCTTG ACATTGAATA AGAAGGCTGA TATAATAGCC
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp41

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CATCCGCAAG TTTATTCTTG ACAGCTGAAT GTAGACGTGG TATAATAGTT
AAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp42

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CATTCGTAAG TTTATTCTTG ACACCTGAGA TGAGGCGTGA TATAATAAAT
AAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp44

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CATCGGGTAG TTTATTCTTG ACAATTAAGT AGAGCCTGAT ATAATAGTTC
AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Constitutional promoter"
/label= Cp5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CATGGGGGAG TTTATTCTTG ACATCATCTT CGTAGCCTGG TATACTACAT
GAGTATGTT 59

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CATGTGGGAG TTTATTCTTG ACACAGATAT TTCCGGATGA TATAATAACT
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) **FEATURE:**

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

TATGCGGTAG TTTATTCTTG ACATGACGAG ACAGGTGTGG TATAATGGGT
CTAGATTAGG 60

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) **HYPOTHETICAL: YES**

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) **FEATURE:**

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CATTCTTTAG TTTATTCTTG ACAAACGTAT TGAGGACTGA TATAATAGGT
GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CATAGTCTAG TTTATTCTTG ACACGCGGTC CATTGGCTGG TATAATAATT
TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 177 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 8..177

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GAATTCGTGA CTCAAACGGG TGGTCGACGG GTGGTTCCAA TTAATTGGCG
TCCCTCTTAT 60

AAAGGCGAGG GTACGTGCGA CAATTGGTAG AGCGAGCGGG GCTCTTAAGT
GCAAGTGACT 120

GCGAACATTT TTTTCGTTTG TTAGAATAAT TCAAGAATCG CTACCAATCA
TGGATCC 177

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 182 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:8..181
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp112

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GAATTCGTGA CTCACGGCAT CTGATGGTTG ACCATAGTCA GGAACATTGT
GCTGGAGTTC 60

CTTGAGGAAT GAGTTATAAA ATGGGAGGTT GCGGCTAATG CCAGGCAGGA
GAGGAACCCT 120

CTTAAGTGCA AGTGACTGCA AACATTTTTT TCGTTTGTTG AATCGCTACC
AATCATGGAT 180

CC 182

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:8..181
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GAATTCGTGA CTCACTAGGC AGGTCACGTT GGCTCTTCGC GGCGCAGGTT
CGTATGCCGC 60

GCCGCCAGGG GCTTTATAAA GGTCGTCCTG GGTACAGTTG GGATGGCTCC
ACGTTTCGGC 120

TCTTAAGTGC AAGTGACTGC GAACATTTTCG TTTGTTAGAA TAATTCAAGA
ATCGCTACCA 180

ATCATGGATC C 191

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 167 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..167
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GAATTCGTGA CTCAGGGCCG TACTAAGTAG CTTTCGTATG CTATGCGGGG
TTTTATAAAT 60

CTTTGGGCCA TGGTCTTGCT GGAAAACACC TCTCTTAAGT GCAAGTGACT
GCGAACATTT 120

TTTTCGTTTG TTAGAATAAT TCAAGAATCG CTACCAATCA TGGATCC
167

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..191
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp154

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

GAATTCGTGA CTCACCGCTC GGGTGCAGGG CCAAGGCGGC GGAATGTGCG
GGGCGTTCTA 60

GCGCAATCGG GGTATAAATT TATAAGGAGG CTGCGGGTGC TAGTTTGTCT
AGTTTGACTC 120

TTAAGTGCAA GTGACTGCGA ACATTTTTCG TTTGTTAGAA TAATTCAAGA
ATCGCTACCA 180

ATCATGGATC C

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..190
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GAATTCGTGA CTCAGGATTA GCTATGCCGG TTGGGATAAG CGAACAAC TG
GAGGTGAGAA 60

GCTTTTTTGTC AGAATATAAA CCCGTTAGTC AGGGTTTGGT GGGATAGGGG
GTACTGTACC 120

TCTTAAGTGC AAGTGACTGC GAACATTTTT TTCGTTTGTT AGAATAATTC
AAGAATCGCT 180

ACCAATCATG GATCC

195

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 179 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 8..179

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp183

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

GAATTCGTGA CTCACTAAGG GTTCGCCATT AACAGAATCG CTGGTAGAAC
ATCGGTAGTT 60

AGGCACCCGA GTATAAACAG GCGGACCCCT CACGGATATC AGCTGATAGT
GCGAGCCTCA 120

ATGCGAACAT TTTTTCGTT TGTTAGAATA ATTCAAGAAT CGCTACCAAT
CATGGATCC 179

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 195 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:8..190

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION:/evidence= EXPERIMENTAL

/standard_name= "Yest promoter"

/label= Yp190

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GAATTCGTGA CTCAGTATCC ACGGGTGTTT GAGGGCTGGT CGCAGGTTAG
CAGGCGAGGG 60

CGGGTGGTTA CGGCTATAAA TGAGTGTTTG CAGCCGGGTA CGGGCGTACG
AGTAGTGATC 120

TCTTAAATGC AAGTGACTGC GAACATTTTT TTCGTTTGTT AGAATAATTC
AAGAATCGCT 180

ACCAATCATG GATCC

195

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 193 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

- (B) LOCATION:8..189
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yest promoter"
/label= Yp191

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

GAATTCGTGA CTCAATGCTG CGGGCGGCAG GAGTCTGGTG TAACTTCCCA
TTTTGAGTGA 60

AAGACAGACC ATCTATAAAC ATTTGGTGGG CAAAGTGGCC TGGCGGATTT
GTTTGGACTC 120

TTAAGTGAAA GTGACTGCGA ACATTTTTTTT CGTTTGTTAG AATAATTCAA
GAATCGCTAC 180

CAATCATGGA TCC 193

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 166 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:8..166
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp192

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GAATTCGTGA CTCACTTAAG GCTACTGCGG AAGTTTAGAT CTAAGGTCGG
AAATAATTTA 60

GAAAATTACG ACATTATAAA TAGCGGAGAG GCCAGGTGAT GGGCACCATT
GTGGGGGGGC 120

TCTTAATTGT TAGAATAATT CAAGAATCGC TACCAATCAT GGATCC
166

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..190
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp212

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GAATTCGTGA CTCAGTCGCC CGCAAGATGG GATGGTGCAT TTAAACACC
CGAATTATAC 60

TCGTCAACTT ATAGTATAAA CGGAACGCGA CGATACGTTC TAGTTTTTCGG
CGAAGTCGAC 120

TCTTAAGTGC AAGTGACTGC GAACATTTTT TTCGTTTGTT AGAATAATTC
AAGAATCGCT 180

ACCAATCATG GATCC

195

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..183
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GAATTCGTAC TCACGACAGC GTTATGACTT CGAGGACCAG CTACTTCCGG
TCGCGTACTA 60

GTTTTTACCT GTATAAACTT TGCTACCGCT GGGCCTTGGT GGTGCTGTCC
CGCTCTTAAG 120

TGCAAGTGAC TGCGAACATT TTTTTCGTTT GTTACAATAA TTCAAGAATC
GCTACCAATC 180

ATGGATCC

188

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..190
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp435

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GAATTCGTGA CTAAATGGAT AAGGTTATCG CCATCACGGA GTCTTCTCTC
ACGTCTGGAG 60

CAGAGGCTAG ACCTTATAAA TTATACATGG TGGGAGAGGC GATAGTCTTT
AGAGACGTGC 120

TCTTAAGTGC AAGTGACTGC GAACATTTTT TTCGTTTGTT AGAATAATTC
AAGAATCGCT 180

ACCAATCATG GATCC

195

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 189 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 8..184

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL

/standard_name= "Yeast promoter"

/label= Yp68

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GAATTCGTGA CTCACAAGAA TGTGGGCGGG TCGTTAACT GAGCCTGGAC
ACCTTGGCTG 60

CGTCGCTTTC GTATAAAGAT CTTAGAGCTG TGGAGTCTGG GTCGAGTGGC
CAGCTCTTAA 120

ATGCAAGTGA CTGCGAACAT TTTTTCGTT TGTTAGAATA ATTCAAGAAT
CGCTACCAAT 180

CATGGATCC

189

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 195 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 8..190

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL

/standard_name= "Yeast promoter"

/label= Yp89

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GAATTCGTGA CTCACTCGGA AGATTGGGTT TACGATTAGG ATGGCGCGGC
AGAACCGGGG 60

GGGATTCCCT TCTATATAAA GGGTTCCGAT ACTACGTGCT GCGGACGGCC
GATCGAGTTA 120

TCTTAAGTGC AAGTGACTGC GAAAATTTTT TTCGTTTGTT AGAATAATTC
AAGAATCGCT 180

ACCAATCATG GATCC 195

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 176 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
(B) LOCATION:8..171
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GAATTCGTGA CTCATCTAGT GAGAGGAGCC GTGGTATCTT GTGTCACCAC
CAGGGGAAAA 60

TAATGGCAGG GGTGTATAAA TGGTCGAGTA GTCGCGACCC ACGCTGCAAG
GCAAGGAACT 120

CTTAAATTTT TTTCGTTTGT TAGAATAATT CAAGAATCGC TACCAATCAT
GGATCC 176

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09/242657

Attorney Docket No. 55411.000002

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of :)
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Peter Ruhdal JENSEN et al.) Group Art Unit: To Be Assigned
)
Serial No.: To be Assigned) Examiner: To Be Assigned
)
Filed: Herewith)

For: ARTIFICIAL PROMOTER LIBRARIES FOR SELECTED ORGANISMS
AND PROMOTERS DERIVED FROM SUCH LIBRARIES

**REQUEST TO EXAMINE APPLICATION ON THE BASIS OF THE
TEXT AMENDED UNDER ARTICLE 34 IN THE PCT**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants request that the examination of the enclosed application be conducted on the basis of the text amended Under Article 34 of PCT. Enclosed are copies of August 26, 1998 response to the first written opinion and of November 26, 1998 response to a second written opinion containing the amendments. Copies of the amendments are also enclosed with the International Preliminary Examination Report dated January 15, 1999. All these documents are enclosed with the Form PTO-1390.

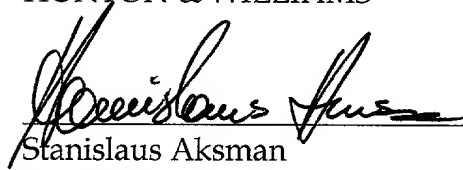
The details of these amendments are listed on the enclosed Form PTO-1390.

Respectfully submitted,

HUNTON & WILLIAMS

Date: February 19, 1999

By:



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09/242657

Artificial promoter libraries for selected organisms and
promoters derived from such libraries.

FIELD OF THE INVENTION

5

This invention concerns artificial promoter libraries and a method of constructing an artificial promoter library for a selected organism or group of organisms. The invention also concerns the individual novel promoters derived from such libraries. Further, the invention concerns a method of optimizing the expression of a gene in a selected organism by use of promoters from such an artificial promoter library for that organism. In principle, artificial promoter libraries according to the invention can be constructed for use in any living organism, but presently they have mostly been of value for modulating gene expression of microorganisms. In connection with this invention the term "microorganism" shall be taken broadly to include prokaryotic organisms such as bacteria as well as eukaryotic microorganisms such as yeasts, other fungi and cell lines of higher organisms.

BACKGROUND OF THE INVENTION

25 Metabolic engineering of living organisms is still in its infancy with respect to industrial applications, despite the fact that genetic engineering has now been feasible for more than a decade. To a large extent, this may be due to the disappointing outcome of many of the attempts so far to improve strain performance. There are at least two reasons for the negative outcome of the attempts to increase metabolic fluxes:

One is that the genetic engineer tends to overlook the subtlety of control and regulation of cellular metabolism. The expression of enzymes that are expected to be rate limiting are increased 10 to 100 fold, e.g. by

placing the gene on a high copy number plasmid. Or, a branching flux in a pathway is eliminated by deleting a gene. Quite often, this will have secondary effects on the metabolism, for instance by lowering metabolite concentrations that are essential to other parts of the cellular metabolism (e.g. processes that are essential to the growth of the organism) and the net result may be that the overall performance of the cell with respect to the desired product is decreased. Instead, it is necessary to tune the expression of the relevant gene around the normal expression level and determine the optimal expression level, for instance as the level that maximizes or minimizes the flux.

The second reason for the negative outcome lies in the rate limiting concept itself: both metabolic control theory (Kacser and Burns, 1973) and experimental determinations of control by individual steps in a pathway (Schaaff *et al.*, 1989; Jensen *et al.*, 1993) have shown that reaction steps which were expected to be rate limiting with respect to a particular flux, turned out to have no or very little control over the flux. Instead, the control and regulation of the cellular metabolism turned out to be distributed over several enzymes in a pathway, and it may be necessary to enhance the expression of several enzymes in order to obtain a higher flux.

According to metabolic control theory, the total flux control exerted by all the enzymes in a pathway, should always sum up to 1. Therefore, after one enzyme concentration has been optimized, the flux control will have shifted to another enzyme(s), and it may then be useful to perform additional rounds of enzyme optimization in order to increase the flux further.

In summary, flux optimization requires 1) fine-tuning of enzyme concentration rather than many fold overexpression

and often 2) optimization of the level of several enzymes in a pathway rather than looking for the rate limiting step.

5 There are now many systems available that allow one to increase the gene expression more than 1000 fold and/or to turn on gene expression at a particular time point during a fermentation process (e.g. using temperature inducible systems). With respect to tuning the steady state
10 gene expression in the fermenter, to say 150% or 70% of the normal expression level, it becomes more difficult. In principle, one could use a *lac*-type promoter in front of the gene of interest, and then add a certain amount of an inducer of the *lac* system, for instance IPTG
15 (isopropyl- β -D-thiogalactoside), or use a temperature sensitive system at the correct temperature. These possibilities are often not practical for large scale industrial applications. The alternative is to use a promoter that has exactly the right strength. However, such pro-
20 moters are seldom available, and furthermore one needs a range of promoter activities in order to optimize the expression of the gene in the first place, see below.

During the past two decades, much work has been done to
25 define and optimize the consensus sequences of microorganisms. In many prokaryotes, one often finds two more or less conserved DNA sequences at approximately position -10 and -35 relative to the start site for transcription, TATAAT and TTGACA, respectively, with approximately 17
30 basepair between the two. The dogma in this field is that, by including these elements, the resulting promoters would tend to become strong. Indeed, promoter up mutations, which are relatively rare events, ususally results in a better match to the above consensus sequences,
35 while down mutations results in a poorer match to the consensus sequences or a less optimal distances between

these. In addition, when random DNA sequences are cloned in place of one of the two consensus sequences, the strength of the resulting promoters usually correlate with the degree of homology to the consensus sequences.

5

In principle, modulation of the strength of promoters could then be achieved by basepair changes in the consensus sequences or by changes in the length of the spacer between these. But the impact of such changes on the promoter strength will tend to be large (see example 1 of this invention), and it is therefore not feasible to achieve small steps of strength modulation through basepair changes in the consensus sequences.

15 While the *length* of the spacer separating the two consensus sequences is known to play an important role for the strength of a promoter, the *sequence* of the spacers between the consensus sequences has usually been considered to be of little importance for the strength of the promoters, and attempts to identify additional consensus sequences in the spacer region through mutagenesis have indeed been unsuccessful. So far, nobody has attempted to randomize the spacer, while keeping the consensus sequences and the spacer length relatively constant.

25

Numerous experiments have been carried out in order to define and optimize the consensus sequences of microorganisms, including experiments where at least one of the consensus sequences was being randomized. In some of these experiments a part of the nucleotides surrounding the consensus sequences was also randomized in order to allow for the generation of promoters with length of spacers different from 17 bp, and/or in order to find possible new consensus motifs around the consensus sequences. The chances that this will generate an efficient

35

promoter is very small and a selection must be applied in order to find those rare cases where the homology to the consensus sequence is sufficiently high to result in even a weak promoter.

5

OBJECT OF THE INVENTION

The promoter libraries that we are aiming at should cover the entire range of promoter activities that could become
10 of interest for engineering of a particular species, preferably from the weakest promoters detectable, to the strongest promoters possible. Moreover, we aim at covering this broad activity range in small steps, say increase in activity by 50-100% per step in order to be
15 suitable for the purpose of flux optimization as described above.

In this invention, we show that the sequence of the spacers between the consensus sequences are far more important than it has previously been appreciated. The spacer
20 sequence can have a strong impact on promoter strength, when 1) a major part of the spacer sequence is varied simultaneously and in a random manner and 2), at the same time, at least half of the consensus sequences are kept
25 constant. We show that if these two conditions are fulfilled, our method can be used to generate promoters that cover wide ranges of activities, including very strong promoters. The range of promoter activities is covered in small steps of activity change which makes these promoters
30 very suitable for metabolic engineering purposes. In addition, we show that the method can be used to generate promoters for a wide range of organisms and at an unusual high frequency.

SUMMARY OF THE INVENTION

The present invention provides an artificial promoter library for a selected organism or group of organisms, comprising a mixture of double stranded DNA fragments the sense strands of which comprise at least two consensus sequences of efficient promoters from said organism or group of organisms, or parts thereof comprising at least half of each, and surrounding or intermediate nucleotide sequences (spacers) of variable length in which at least 7 nucleotides are selected randomly among the nucleobases A, T, C and G, with the proviso that previously known promoter sequences and promoter sequences isolated from natural sources are not comprised.

The broadest variation in promoter strengths is obtained when at least 10, preferably at least 12, and more preferably at least 14, nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

The sense strands of the double stranded DNA fragments may also include a regulatory DNA sequence imparting a specific regulatory feature to the promoters of the library. Such specific regulatory feature is preferably activation by a change in the growth conditions, such as a change in the pH, osmolarity, temperature or growth phase.

For cloning purposes the double stranded DNA fragments usually have sequences comprising one or more recognition sites for restriction endonucleases added to one of or both their ends; most conveniently sequences specifying multiple recognition sites for restriction endonucleases (multiple cloning sites MCS).

The selected organism or group of organisms may be selected from prokaryotes and from eukaryotes, in particular from prokaryotes and eukaryotic microorganisms such as yeasts, other fungi and cell lines of higher organisms.

An interesting group of prokaryotes i.a. in the dairy industry consists of lactic acid bacteria of the genera *Lactococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillus* and *Leuconostoc*, in particular strains of the species *Lactococcus lactis* and *Streptococcus thermophilus*. Other interesting prokaryotes are bacteria belonging to the genera *Escherichia*, *Bacillus* and *Pseudomonas*, in particular the species *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas putida*.

In an artificial promoter library for prokaryotes said consensus sequences may for example comprise the -10 signal (-12 to -7): TATAAT and at least one activator protein binding site upstream of the -10 signal or parts thereof comprising at least 3 conserved nucleotides of each.

Most often the consensus sequences to be retained in an artificial promoter library for prokaryotes will comprise the -35 signal (-35 to -30): TTGACA and the -10 signal (-12 to -7): TATAAT or parts of both comprising at least 3 conserved nucleotides of each.

More efficient promoters are usually obtained when said consensus sequences comprise from 4 to 6 conserved nucleotides of the -35 signal and from 4 to 6 conserved nucleotides of the -10 signal, preferably 5 or 6, and more preferably all 6 nucleotides of each. The most efficient promoters are obtained when said consensus sequences further comprise intervening conserved motifs, e.g. selected

from the conserved motifs -44 to -41: AGTT, -40 to -36: TATTC, -15 to -14: TG, and +1 to +8: GTACTGTT.

In such promoters the length of the spacer between the -35 signal and the -10 signal should be 14-23 bp, preferably 16-18 bp, and more preferably 17 bp. This should be understood to mean the spacer length between the hexamer signals, even when some of the nucleotides in the signals have been mutated.

In eukaryotic organisms said consensus sequences should comprise a TATA box and at least one upstream activation sequence (UAS).

An interesting eukaryotic microorganism is the yeast species *Saccharomyces cerevisiae*, normal baker's yeast. In promoters to be used in *Saccharomyces* the consensus sequences may further comprise a transcription initiation signal (TI box) functioning in *Saccharomyces cerevisiae*.

In a specific embodiment of an artificial promoter library according to the invention for *Saccharomyces cerevisiae* said consensus sequences comprise the TATA box: TATAAA, the UAS_{GCN4p}: TGA CTCA, and the TI box:

CTCTTAAGTGCAAGTGACTGCGA, which also functions as the binding site for the arginine repressor, *argR*.

The individual promoters of the artificial promoter libraries defined above are also comprised by the invention. Specific promoters which have been constructed according to the following examples are those stated in the SEQ IDs Nos. 5 to 58 below. The invention further comprises artificial promoters which are derived from promoters defined by the artificial promoter libraries of the invention.

The present invention also provides a method of constructing an artificial promoter library for a selected organism or group of organisms, which comprises selecting at least two consensus sequences of efficient promoters from said organism or group of organisms; synthesizing a mixture of single stranded DNA sequences comprising said consensus sequences, or parts thereof comprising at least half of each, and surrounding or intermediate nucleotide sequences (spacers) of variable length in which at least 7 nucleotides are selected randomly among the nucleobases A, T, C and G; and converting the single stranded DNA sequences into double stranded DNA fragments by second strand synthesis.

As previously mentioned, the broadest variation is obtained when at least 10, preferably at least 12, and more preferably at least 14, nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

In order to obtain an artificial promoter library which is susceptible to regulation, the single stranded DNA sequences which are synthesized may include a regulatory DNA sequence imparting a specific regulatory feature to the promoters of the library. Such specific regulatory feature is preferably activation by a change in the growth conditions, such as a change in the pH, osmolarity, temperature or growth phase.

Also, in order to obtain an artificial promoter library suitable for cloning, a sequence specifying one or more recognition sites for restriction endonucleases may be added to one of or both the ends of the single stranded DNA sequences in the synthesis, or a linker comprising such restriction sites may be ligated to one of or both the ends of the double stranded DNA fragments. Most conveniently such sequences specify multiple recognition

sites for restriction endonucleases (multiple cloning sites MCS).

5 The selected organisms for which artificial promoter libraries can be prepared by the method according to the invention and the various degenerated sequences to be chosen for the promoter libraries of specific organisms are the same as discussed above for the artificial promoter libraries per se.

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With respect to possible uses of the artificial promoter libraries described above, the invention further provides a method of optimizing the expression of a gene in a microorganism, which comprises

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a) selecting a set of promoters covering a range of promoter activities in relatively small steps of activity change from an artificial promoter library according to any one of claims 1-26 or constructed by the method according to any one of claims 30-55;

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b) cloning said set of promoters into said organism placing in each clone said gene under the control of at least one promoter from the set;

25

c) growing the selected clones and screening them to find the one showing optimized flux of product formation.

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This method is preferably used with organisms selected from the group consisting of prokaryotic and eukaryotic microorganisms such as bacteria, yeasts, other fungi and mammalian cell lines.

In other aspects, the invention pertains to a method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

5

(i) constructing, using the above method of constructing a promoter library, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the
10 gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

15 (iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation,

and a promoter sequence that is capable of optimising the expression of a gene in a selected organism, which promoter sequence is obtainable by the above method of
20 isolating a promoter sequence.

AMENDED SHEET

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. A library of artificial promoters for *L. lactis*, from example 1. The promoter activities (Miller units) were assayed from the expression of a reporter gene (*lacLM*) encoding β -galactosidase transcribed from the different synthetic promoter clones, on the promoter cloning vector pAK80, in strain MG1363, grown in GM17 medium supplemented with 2 μ g/ml erythromycin. The patterns of the data points indicate which promoter clones differed in either the -35 or the -10 consensus sequence, or in the length of the spacer between these two consensus sequences.

Figure 2. A library of artificial heatshock regulated promoters for *L. lactis*, from example 2. The promoter activities (Miller units) were assayed from the expression of a reporter gene (*gusA*) encoding β -glucuronidase, transcribed from the different synthetic promoter clones, on the chromosome in strain LB436. The cells were grown in GM17 medium supplemented with 2 μ g/ml erythromycin, and at two different temperatures, 30 and 37 °C. The assay for β -glucuronidase was parallel to the β -galactosidase assay (see example 1) except that X-gluc was used as the substrate.

Figure 3. The library of artificial promoters from example 1, assayed for activity in *E. coli*. The promoter activities (Miller units) were assayed from the expression of a reporter gene (*lacLM*) encoding β -galactosidase transcribed from the different synthetic promoter clones, on the promoter cloning vector pAK80, in strain BOE270, grown in LB medium supplemented with 200 μ g/ml erythromycin. The patterns of the data points indicate which promoter clones differed in either the -35 or the -10 con-

sensus sequence, or in the length of the spacer between these two consensus sequences.

Figure 4. A library of artificial promoters for *S. cerevisiae*, from example 7. The promoter activities (Miller units) were assayed from the expression of a reporter gene (*lacZ*) encoding β -galactosidase transcribed from the different synthetic promoter clones, on the promoter cloning vector pYLZ-2 in *S. cerevisiae* strain SG24 (URA3-52), grown in SD minimal medium supplemented with 2% glucose. YP24 and YP435 has a 1 bp deletion and a point mutation, respectively, in the UASGCN4p binding site. pTK101 contains a promoter in which the UAS sequence has been deleted and the TATA box is present.

Figure 5. Regulation of artificial yeast promoters by external arginine, from example 7. The promoter activities (Miller units) were assayed from the expression of a reporter gene (*lacZ*) encoding β -galactosidase transcribed from the different synthetic promoter clones, on the promoter cloning vector pYLZ-2 in *S. cerevisiae* strain SG24 (URA3-52), grown in SD minimal medium supplemented with 2% glucose, with (SDG) or without 1 g/l arginine (SDG+arg). YP183 has a 13 bp deletion in the binding site for the argR repressor.

Figure 6. Regulation of artificial yeast promoters by external amino acids, from example 7. The promoter activities (Miller units) were assayed from the expression of a reporter gene (*lacZ*) encoding β -galactosidase transcribed from the different synthetic promoter clones, on the promoter cloning vector pYLZ-2 in *S. cerevisiae* strain SG24 (URA3-52), grown in SD minimal medium supplemented with 2% glucose (SDG) or complex medium (containing amino ac-

ids) without uracil (SC-ura). YP24 has a 1 bp deletion in the UAS_{GCN4p} binding site.

DETAILED DESCRIPTION OF THE INVENTION

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In our method, degenerated oligonucleotides are synthesized for the organism or group of organisms for which a promoter library should be constructed. The sequences of the oligonucleotides are written by combining the available knowledge from the literature, on the features that makes a promoter function efficiently in that particular organism. The amount of information that needs to be fixed in the oligonucleotide is somewhat variable among different organisms. In *E. coli* for instance, promoters of considerable strength may be formed by less perfect matches in the -35 and -10 consensus sequences and by spacing between these sequences deviating from 17bp (TTGACA and TATAAT respectively), whereas the requirements for strong promoters in *L. lactis* appear to be more strict.

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Secondly, the single stranded oligonucleotides are converted into double stranded DNA fragments and cloned into a promoter probing vector. The promoter-containing clones are identified e.g. by their ability to give colonies with various extents of colour on indicator plates. This should in principle give us only very strong promoters, but we discovered that by allowing the spacer sequences between the consensus sequences to vary in a random manner, the strength of the resulting promoters are modulated. In fact, using this method we obtained promoter libraries, spanning the entire range of promoter activities that is likely to become of interest, in small steps of activity change.

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Optimization of gene expression could then be achieved as follows: 1) From the promoter library one chooses promoters that have e.g. 25%, 50%, 100%, 200% and 400% of the strength of the wild-type promoter. 2) Then, these promoters are cloned in place of the wild-type promoter upstream of the gene of interest. 3) The magnitude of the variable to be optimized (e.g. the flux through a pathway) that is obtained with each of these five constructs is then measured and the optimal construct is used directly as the production organism. It may be necessary to fine-tune the expression further or to expand the range of promoter activities. A direct advantage of this system over the inducible systems described above is that once the optimal promoter activity has been determined, the strain is in principle ready for use in the fermentation process.

In one preferred embodiment the random spacers method of the invention is used for generating a series of constitutive promoters for the Gram-positive bacterium, *Lactococcus lactis*. In other preferred embodiments we show that promoters generated by the random spacers method of the invention are functional in at least two species of Gram-negative bacteria, *Escherichia coli* and *Pseudomonas*, as well as in the Gram-positive bacterium *Bacillus subtilis*. We also show that the strength of the individual promoter is dependent on which organism it is being used in, i.e. that in some organisms a particular promoter is strong, in others it is weak, but in all the organisms tested, the promoters cover a broad range of activities, in small steps of activity change.

Often it is desirable to activate gene expression to a certain extent and at a certain stage of a fermentation, e.g. because the gene product that is expressed inhibits the growth of the cells. It is then useful to combine the

above technique for obtaining promoters of different strength with some regulatory mechanism, e.g. so that the promoter will be activated by a change in the pH, temperature or growth phase.

5

Thus, in another preferred embodiment the random spacers method of the invention is used for generating a series of specifically regulated promoters. As illustrated in Example 2, the above approach is used in combination with specific regulatory DNA sequences to generate a library of heatshock-regulated promoters for the Gram-positive bacterium, *Lactococcus lactis*.

10

In addition to prokaryotes, eukaryotic microorganisms (yeast and other fungi as well as mammalian cell lines,) are important microorganisms for production of a range of organic compounds and various proteins. It is therefore of interest to develop the above approach for modulating gene expression in these organisms as well. Thus, in yet another preferred embodiment, as illustrated in Example 7, the random spacers method of the invention is used for generating a series of promoters for the bakers yeast, *Saccharomyces cerevisiae*. The promoters are here equipped with GCN4p and ARGR regulation.

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The regulation of transcription initiation in the eukaryotic cell is somewhat more complex compared to the prokaryote. The transcription start site is normally preceded by a so-called TATA box that contains the consensus sequence TATAAA or parts hereof, but unlike in the prokaryote, the distance from the TATA box to the transcription start site is much less defined. In *Saccharomyces cerevisiae* this distance is typically 40-120 nucleotides (Oliver and Warmington, 1989). The so-called -35 consensus hexamer which is found in many prokaryotic promoters is absent in *Saccharomyces cerevisiae*. Instead so-called

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35

upstream activation sequences (UAS) are found upstream of the transcription initiation site. These UAS are recognised by specific DNA binding proteins that can then act as activators of transcription initiation. For instance, the UAS sequence that is found upstream of the genes involved in aminoacid biosynthesis, UASGCN4p, consists of a DNA sequence that specifies a binding site for the GCN4 protein, which activates the transcription of these genes (Hinnebusch, 1992). In contrast to prokaryotes, where the distance between the promoter elements appears to be critical for the strength of promoters (see example 1), the distance between the TATA box and the UAS sequence in eukaryotic promoters is highly variable and may be up to about 1000 bp. Some genes even contain more than one copy of the UAS, but one seems to be sufficient for activation.

One of the UAS sequences known from yeast is the binding site for the GCN4 protein. Promoters that contain the binding site for GCN4 protein should be regulated by the status of amino acid supply in the growth medium: in the absence of amino acids, the GCN4 protein is formed and binds specific UAS sequences to stimulate transcription at promoters involved in biosynthesis of aminoacids. The consensus sequence for the GCN4 protein binding site (UASGCN4p) is a short inverted repeat, TGACTCA.

A promoter in *Saccharomyces cerevisiae* that is activated by the GCN4 protein is the ARG8 promoter. In this promoter, there is only one copy of the UASGCN4p sequence, and it is located 59 bp from the TATA box (we refer to this distance as spacer 1). Transcription initiation takes place some 40-60 nucleotides downstream of the TATA box. The ARG8 promoter also contains a DNA sequence that functions as the binding site for the arginine repressor, *argR* (Crabeel et al., 1995), which makes the promoter four fold repressible by external arginine.

Thus, in this case the promoter is located within 136 bp, and it contains two regulatory features, which makes the system attractive for developing a promoter library by the random spacers method of the invention as outlined in the previous examples. But the method is not limited to this model system; in principle, any combinations of TATA boxes, UAS sequences, repressor binding sites etc., separated by spacers smaller than about 1000 nucleotides, should be suitable as a starting point for this method.

EXAMPLE 1

Design of a degenerated oligonucleotide for a *L. lactis* promoter library.

According to the literature (see review in de Vos & Simons, 1994), strong promoters in *L. lactis* tend to have the following nucleotide sequences in common (numbers refer to the position relative to the transcription initiation site, which is given number +1): -12 to -7: TATAAT; -15 to -14: TG; -35 to -30: TTGACA. The spacing between -10 and -35 seems to be 17 nucleotides. However, closer comparison of the promoter sequences that have been published for *L. lactis* reveals that in a number of positions besides the ones mentioned above, nucleotides are more or less well conserved. Some of these positions are: -1: A; -3: A or T (=W); -6: A; -13: A or G (=R); -40 to -36: TATTC. In addition, Nilsson and Johansen (1994, BBA) pointed out two motives, +1 to +8: GTACTGTT, and -44 to -41: AGTT, that appear to be well conserved between relatively strong promoters (promoters for transfer RNA and ribosomal RNA operons) from *L. lactis*. These motives may confer both strength and growth rate dependent expression from the promoter.

When these additional motives are included, one arrives at the following 53 nucleobase degenerated sequence for

an efficient promoter in *L. lactis*. Out of these 53 nucleobases, 34 bases are conserved, two are semi-conserved (R and W) and 17 are allowed to vary randomly between the four nucleobases.

5

5' AGTTTATTCTTGACANNNNNNNNNNNNNNTGRTATAATANNWNAGTACTGTT 3'

In addition, this degenerated sequence is flanked by sequences that specify multiple recognition sites for restriction endonucleases (multiple cloning site MCS), and the actual oligonucleotide mixture to be synthesized has the following degenerated sequence reported in SEQ ID No. 1:

15

MboI
DpnI
AlwI
NlaIV
BstYI
BamHI MseI
AlwI AflIII SspI NsiI

20

1
5'CGGGATCCTTAAGAATATTATGCATNNNNNAGTTTATTCTTGACANNNNNNNNNNNNNNT

25

AluI
PvuII
NspBII

30

SfcI
MseI Fnu4HI
RsaI HpaI PstI
ScaI HincII BbvI EcoRI

61
GRTATAATANNWNAGTACTGTTAACTGCAGCTGAATTCGG 100
3'

35

A mixture of oligonucleotides according to this specification was synthesized by Hobolth DNA synthesis.

40

This oligonucleotide mixture is single stranded initially and must be converted into double stranded DNA fragments for the purpose of cloning. This was done by synthesizing in vitro a 10 bp oligonucleotide, having a sequence complementary to the 3' end of the promoter oligonucleotide. This oligonucleotide was then used as a primer for second strand synthesis by the Klenow fragment of DNA polymerase

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I in the presence of dATP, dCTP, dGTP and dTTP. In this way the second DNA strand became exactly complementary to the first DNA strand.

5 The result is a mixture of 100 bp double stranded DNA fragments containing multiple recognition sites for restriction endonucleases in both the 3' and 5' end. These DNA fragments were then cut with restriction endonucleases in order to create suitable "sticky" ends compatible
10 with the ends of the vector DNA fragment, pAK80 (Israelson *et al.*, 1995). pAK80 is a shuttle vector, meaning that it has replication origins for propagation in both *E. coli* and *L. lactis*. In this way, the cloning steps can be conveniently performed in *E. coli*, while the
15 subsequent physiological experiments can be done in *L. lactis*. Furthermore, pAK80 carries a promoterless β -galactosidase reporter gene system (*lacLM*) downstream a multiple cloning site. Thus, pAK80 does not express the *lacLM* genes, unless a promoter is inserted in the multiple cloning site.
20

Two cloning strategies were used for cloning the mixture of double stranded DNA fragments into the cloning vector pAK80:

- 25
- 1) The mixture was digested with *BamHI* and *PstI* and the vector pAK80 with *PstI* and *BglII* (*BglII* is compatible with *BamHI*).
 - 30 2) The mixture was digested with *SspI* and *HincII* and the vector pAK80 with *SmaI* (all three enzymes produce blunt end DNA fragments).

In both cases the vector DNA was further treated with
35 Calf Intestine Phosphatase (CIP) to prevent religation of the cloning vector. Subsequently, the fragment mixture

and vector DNA were ligated overnight at 16 °C using T4 DNA ligase and standard ligation conditions.

Ligation mixtures were transformed into *E. coli* K-12 Δ lac, with selection for erythromycin resistance. Cells of the *E. coli* K-12 strain, MT102, were made competent using standard treatment with Ca^{++} ions (Maniatis et al., 1982). Ligation mixtures were then transformed into these cells using a standard transformation procedure (Maniatis et al., 1982), and the resulting clones were screened for β -galactosidase activity that will produce blue colonies on plates containing X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside). The transformation mixture was plated on LB plates containing 200 $\mu\text{g/ml}$ erythromycin, 1% glycerol and 100 $\mu\text{g/ml}$ X-gal. 150 erythromycin resistant transformants were obtained, all white initially, but after prolonged incubation (two weeks at 4 °C), 46 of these colonies had become light blue. Thus, using strategy 1) we found 17 blue colonies (CP30 to CP46), and using strategy 2) we found 29 blue colonies (CP1 to CP29).

Plasmid DNA was isolated from each of these clones (CP1 to CP46) and analysed by restriction enzyme mapping. Nearly all plasmids (except CP31 and CP43) contained promoter fragments inserted into the MCS of pAK80, in the orientation that would direct transcription of the otherwise promoterless *lacLM* genes on this vector.

These 46 plasmid DNA preparations were then transformed into *L. lactis* subspecies *lactis* MG1363 with selection for erythromycin resistance. Cells of the *L. lactis* subspecies *lactis* strain, MG1363 (Gasson, 1983) were made competent by growing the cells overnight in SGM17 medium, containing 2% glycine, as described by Holo and Nes (1989). Plasmid DNA from each of the 46 clones described above was then transformed into these cells using the electroporation procedure (Holo and Nes, 1989). After re-

generation, the cells were plated on SR plates containing 2 µg/ml erythromycin. Subsequent screening for blue color on X-gal plates revealed large differences in β-galactosidase activity between the 46 clones; some clones
5 gave dark blue colonies after 24 hours of incubation, others only light blue colonies after more than 1 week of incubation.

The β-galactosidase activities of liquid cultures of the
10 46 clones in MG1363 were then determined as described by Miller (1972) and modified by Israelsen *et al.* (1995). Cultures of the strain MG1363, each carrying one of the 46 plasmid derivatives of pAK80, were grown in M17 medium supplemented with 1% glucose overnight at 30 °C. 25-100
15 µl of these cultures were then used in the subsequent β-galactosidase assay, except in the case of the weakest promoter clones, where 2 ml of culture was used (after 20 fold concentration by centrifugation). These results are shown in Figure 1. Apparently, there are very large dif-
20 ferences in the efficiency of the cloned promoter fragments, and together these clones cover a range of promoter activities from 0.3 units of β-galactosidase activity to more than 2000 units, which is probably the strongest promoters known for this organism

25 In addition, the broad range is covered by small changes in activity and, therefore, these promoter fragments will allow us not only to obtain a wide range of expression of genes in *L. lactis*, but also to tune the expression of
30 genes in *L. lactis* in small steps for the purpose of flux optimization.

DNA sequencing of the 46 clones described above revealed that most of the inserted promoter fragments had the DNA
35 sequence that was originally specified for the oligonucleotide design (see above), whereas the sequence of the remaining fragments deviated slightly from that sequence.

Most of the promoter fragments that gave the lower activities in the β -galactosidase assay (70 units of β -galactosidase or less) had either an error in one of the consensus sequences or a 16 bp spacer between the consensus sequences. This result is in accordance with the dogma, i.e. that changes in the consensus sequences have strong effects on the activity of a given promoter, and emphasizes the fact that a more subtle approach is needed in order to generate a promoter library that covers a range of activities in small steps of activity change. Clearly, if we would have allowed only changes in the consensus sequences and/or changes in the length of the spacer, instead of allowing the sequence of the spacers to vary randomly, only fairly weak promoter clones would have resulted, and the resulting library would not be suitable for metabolic engineering.

In general, the clones that gave high activities (more than 70 units) had the same sequence as specified by the oligonucleotide. In total, the activity of the clones that had intact consensus sequences and 17 basepair spacer length, spanned activities from 5 units (CP4) to 2000 units (CP25). These results therefore show that at least a 400 fold change in promoter activity can be obtained by randomizing the spacer while the consensus sequences are kept constant.

Usually, for metabolic engineering purposes, relatively strong promoters are desired. However, there may also be cases where rather weak promoters are needed. The relatively few errors that had occurred during synthesis of the above oligonucleotide mixture, were not intended to be present in the promoter fragments; and our data then suggest that it may be useful to generate, deliberately, a mixture of oligonucleotides which have a low percentage of errors in the consensus sequences.

Enzymes used in the various enzymatic reactions above were obtained from and used as recommended by Pharmacia and Boehringer.

5 EXAMPLE 2

Design of a degenerated oligonucleotide for a library of temperature regulated *L. lactis* promoters.

10 This example illustrates the development of a temperature regulated promoter library for *L. lactis*. A regulatory element comprising an eight basepair inverted repeat that has been shown to be involved in the heatshock response of *L. lactis* is inserted a few base pairs upstream of the
15 -35 sequence. The minimal extent of such a regulatory element seems to be 27 basepairs:

5'-TTAGCACTCNNNNNNNNNGAGTGCTAA-3'

IR spacer IR

20 containing a 9 bp (or longer) inverted repeat (IR) separated by 9 (or fewer) basepairs. It should therefore be possible to combine this inverted repeat with the approach for obtaining constitutive promoters of different
25 strength and thus obtain a series of promoters with various basal activities which can be induced several fold by changing the temperature of the culture medium.

Therefore, an oligonucleotide was designed, which includes the core part (from position -35 to +6) of the sequence from the constitutive promoter series above (see example 1 and SEQ ID No. 1). The sequence upstream of the -35 hexamer has been replaced by the above inverted repeat sequence, and the sequence downstream of position +6
30 has also been modified, eliminating two conserved regions compared to example 1 (+1 to +8: GTACTGTT and -44 to -41: AGTT, which have been implicated in growth rate regula-

tion, but which turned out to be dispensable with respect to creating strong promoters for *L. lactis*, see example 1). The sequence of the spacer (sp.1) between the two inverted DNA sequences in the inverted repeat was here allowed to vary randomly in order to see whether this had any effect on the temperature regulation of the resulting promoters, e.g. how many fold they could be induced by changing the temperature. The importance of the spacing (sp.2) between the inverted repeat and the -35 hexamer is not known, but in principle this region may contribute to or modulate the heatshock response of promoters. In order to limit the number of parameters, however, we have chosen here to include a naturally occurring configuration (derived from the *dnaJ* promoter from *L. lactis*; van Asseldonk et al., 1993): a short spacer sequence consisting of 5 times T.

When these sequences are combined, one arrives at the following 73 bp consensus sequence for a temperature regulated promoter in *L. lactis*. Out of these 73 bp, 45 are conserved, two are semi-conserved (R and W) and 26 are allowed to vary randomly between the four nucleobases.

5' TTAGCACTCNNNNNNNNNGAGTGCTAATTTTTTTGACNNNNNNNNNNNNNNNTGR
 IR spacer 1 IR sp.2 spacer 3

TATAATANNWNAGTACTG 3'

In addition, this degenerated sequence was flanked by sequences that specify multiple recognition sites for restriction endonucleases (multiple cloning sites MCS), and the actual oligonucleotide mixture that is being synthesized has the following degenerated sequence reported in SEQ ID No. 2:

```

      MboI
      DpnI
      AlwI
      NlaIV
5      BstYI      MseI  MseI
      BamHI  AluI  SspI
      AlwI  HindIII  AseI
      1
10  5'CGGGATCCAAGCTTAATATTAATTAGCACTCNNNNNNNNNGAGTGCTAATTTTTTTTGACA
      IR                                IR                                -35

      AluI
      PvuII
      NspBII
15      SfcI      EcoRI
      PstI      ApoI
      RsaI  Fnu4HI  MaeI
      ScaI  BbvI  XbaI
20  61      A      T      113
      NNNNNNNNNNNNTGGTATAATANNANAGTACTGCAGCTGTCTAGAATTCGG 3'
      -15  -10      +1

```

This oligonucleotide mixture was converted into double stranded DNA fragments (DSDF) and cloned into the promoter cloning vector pLB85i. pLB85i has an origin for replication in *E. coli* but not in *L. lactis* and selectable markers for both organisms. Instead of an origin of replication for *L. lactis*, it contains the *attP* sequence which can direct the insertion of the plasmid into the *L. lactis* chromosome if the *int* gene product is supplied *in trans*. This example therefore also serves to illustrate the modulation of chromosomally encoded genes, using promoters generated through the random spacers method. pLB85i is also a promoter cloning vector and contains a multible cloning site upstream of a promoterless *gusA* gene. *gusA* is similar to the *lacZ* and *lacLM* screening sytems used in the other examples, except that the substrate is X-gluc instead of X-gal. It was chosen as the reporter gene for this particular application, where heatshock regulated promoters are supposed to be analysed. This was because the *gusA* gene product is not heat labile, which seems to be a problem connected with the *lacLM* gene used in example 1.

The DSDF mixture was here digested with *XbaI* and *AseI* and the vector pLB85i was digested with *XbaI* and *NdeI* (*NdeI* is compatible with *AseI*) and further treated with alkaline phosphatase to remove the 5 phosphate groups from the vector. After ligation of the DFDS mixture and the vector, the ligation mixture was transformed into strain KW1, a *gusA* negative *E. coli* strain, with selection for ampicillin resistance. This resulted in approximately 100 colonies of which 80% had different blue color intensities, indicating that these clones were carrying putative heatshock promoter fragments. 20 blue clones were picked for further analysis. Restriction analysis of plasmid preparations from these clones showed that they had inserts of approximately the right size. Subsequently, 6 of these plasmid preparations were used to transform LB436 (a derivative of *L. lactis* MG1363 which contain a second plasmid, pLB81, supplying the necessary *int* gene for integration of the plasmids into the *attB* site on the *L. lactis* chromosome). From each of the transformations colonies were isolated with putative integrations of the constructs into the *attB* attachment site on the *L. lactis* chromosome. The integration of the constructs was confirmed by standard colony PCR, using one primer in the *attB* region and one primer in the pLB85i region. The clones were subsequently tested for their ability to form blue colonies on GM17 medium supplemented with erythromycin and 100 µg/ml X-gluc at 30 °C and 37 °C, respectively, and the clones showed a clear difference in color intensity at the two temperatures, indicating that the promoter activities were now under temperature regulation. Next the *gusA* expression from the heatshock promoters was measured in liquid cultures at the two temperatures, for 5 selected heatshock-regulated promoters, see Figure 2. The clones had different activities and covered a broad range of promoter activities at 30 °C,

and 4 out of the 5 clones (all except HP6) gave higher promoter activities at 37 °C, which shows that the promoters were indeed temperature regulated. Interestingly, the promoters were regulated by almost the same fold, i.e. from 1.7 to 2.3 fold by the temperature shift from 30 to 37 °C, which indicates that the spacer (spacer 1) in the inverted repeat is of minor importance with respect to determining the fold of induction of these artificial promoters.

10

We have here been looking at the accumulated *gusA* activity, and although the data clearly show that the promoters are temperature regulated, one skilled in the art of analysing gene expression will appreciate that this makes it more difficult to observe the changes in promoter activities, that are brought about for instance by some change in external parameter such as temperature. In addition, it has been shown that the activity of heatshock regulated promoters is temporarily ten fold higher immediately after the temperature shift, than it appears when the steady state levels are compared as they are on Figure 2. To observe the change in promoter activity more carefully, one should look at the *rate* of protein or mRNA synthesis, before and at various times after the perturbation in temperature. We therefore isolated RNA from the 5 clones at various times after the change in temperature from 30 °C to 37 °C and visualized the *gusA* mRNA by standard Northern blotting (Maniatis *et al.*, 1983), to analyze how many fold the individual promoter clone was induced by the temperature change.

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EXAMPLE 3

The Gram-positive bacterium, *Bacillus subtilis* is used extensively as an industrial bioreactor for the produc-

35

tion of a range of heterologous proteins. It was therefore of interest to test whether the random spacers method of the invention could also be used to generate promoter libraries for this organism. The consensus sequences for *Bacillus subtilis* are very similar to the consensus sequences for *E. coli* and *L. lactis*, and we could therefore test whether the approach was also valid for *Bacillus subtilis* by subcloning a number of CP promoters into a promoter cloning vector for this *Bacillus subtilis* and then ask 1) whether the CP promoters are active in *Bacillus subtilis* and 2) whether, also in this organism, the spacer between the consensus sequences plays an important role for the promoter strength. We chose to use the promoter cloning vector, pDG268, which is designed for integration of promoter fusions to *lacZ* into the *amy* locus on the *Bacillus subtilis* chromosome. The vector confers ampicillin and neomycin resistance, and it will replicate in *E. coli* for the initial cloning purposes, but not in *Bacillus subtilis*. When the linearized form of the vector is transformed into *Bacillus subtilis* it will be inserted into the *amy* locus on the *Bacillus subtilis* chromosome. This example therefore also serves as an example of the use of the promoters generated through the random spacers methods for modulation of chromosomally encoded genes.

The CP promoters were indeed active also in *Bacillus subtilis*, and the individual strength of the promoters were again very different from each other. The fact that the promoter library also covered a broad range of promoter activities in *Bacillus subtilis*, shows that the random spacers method is also valid for this organism.

EXAMPLE 4

The CP promoters of example 1 were designed for use in *L. lactis*, but the consensus sequences of *E. coli* promoters are included in the sequence of the oligonucleotide given in example 1. Furthermore, the vector used for generating the CP promoter library in example 1 is a shuttle vector for *L. lactis* and *E. coli*, so this also allowed us to analyse the activity of the CP promoters in the Gram-negative bacterial host, *E. coli*. Figure 3 shows the activity of 33 of the CP promoters in *E. coli*. Clearly, the activity of the individual CP promoters is also here very different, and together the promoters cover a broad range of activities. Interestingly, the correlation between the strength of the individual promoters in *E. coli* and *L. lactis* was not very strong: some promoters that were found to be strong promoters in *L. lactis* were found to be weak in *E. coli* and vice versa.

The activity of the promoters, in terms of β -galactosidase activity, was generally much lower than the activity found in *L. lactis*. This was probably due to the fact that the promoter cloning vector, pAK80 is designed and optimised for use in the Gram-positive bacterium, *L. lactis*, and the translation efficiency in *E. coli* could therefore be low. We therefore sub-cloned three of the CP promoters (CP20, CP22 and CP25) into the promoter cloning vector, pCB267, which was designed for cloning *E. coli* promoters in upstream of a promoterless *lacZ* gene, encoding β -galactosidase. In this promoter system, the CP promoters turned out to be very efficient promoters, but the relative difference in strength between the three promoters was conserved. Thus, the CP25 promoter gave 2.5 fold higher activity than the hybrid promoter, *ptac*, which is considered to be among the strongest promoters known for

use in *E. coli*. These data therefore further demonstrate the strength of our approach: by analysing a relatively small number of promoter clones obtained through the random spacers method of the invention, we have managed to arrive at some of the strongest promoters in both *E. coli* and *L. lactis*.

EXAMPLE 5

Bacteria belonging to the Gram-negative species, *Pseudomonas*, are becoming increasingly important due to their application in e.g. biodegradation of chemical waste products in polluted soil. But also here the genetic engineering is hampered by the lack of suitable promoters and expression systems. The literature on *Pseudomonas* promoters revealed that the consensus sequences for *Pseudomonas* are somewhat less well defined than those of *E. coli*, *L. lactis* and *Bacillus subtilis*. Thus, in the -35 region one often finds TTGR conserved (R=A or G) whereas the rest of the -35 consensus sequence is varying between different promoters. The -10 consensus sequence is probably TATRAT. The spacing between the TTGR motive and the -10 sequence is 18-19 bp, which is equivalent of the 16-17 bp spacer often found in *E. coli*. It follows that the consensus sequences for vegetative promoters in this organism are quite close to the consensus sequences for *E. coli* and *L. lactis*.

We therefore tested a range of the CP promoters from example 1 in *Pseudomonas putida* by cloning the promoters into a cloning vector that contains a promoterless β -galactosidase gene. Again the activity of the CP promoters differed in strength over a broad range of promoter activities. These results show that also in this species, the random spacers method of the invention could be used

to generate both relatively strong promoters and also a broad range of promoter activities covered in small steps of activity change

5 **EXAMPLE 6**

We also designed an oligonucleotide based on the consensus sequences stated in example 5 and incorporated multiple cloning sites as described in example 1. The following oligonucleotide was synthesised.

MCS-(N)8-TTGR-N₁₉-TATRAT-(N)8-MCS

The oligonucleotide was converted to double stranded DNA, using a primer homologous to the 3' end of the oligonucleotide and cloned upstream of a promoterless β -galactosidase gene on a promoter probe vector. The ligation mixture was transformed directly into a *Pseudomonas putida* strain with selection for the antibiotics resistance carried by the plasmid and on plates containing X-gal. This resulted in approximately 100 clones of various blue color intensities. Subsequently, 30 clones were analysed for β -galactosidase activity as described above. These results showed that also in this species, the random spacers method of the invention could be used to generate both relatively strong promoters and also a broad range of promoter activities covered in small steps of activity change.

30 **EXAMPLE 7**

Design of a degenerated oligonucleotide for a *Saccharomyces cerevisiae* promoter library.

35 A 199 bp oligonucleotide was designed, which includes, starting from the 5' end: an *EcoRI* restriction site (for

complementary to the last 23 bp of the 3' end of the 199 bp degenerated oligonucleotide as described in example 1. Subsequently, it was cloned into either of the two promoter cloning vectors, pYLZ-2 and pYLZ-6 (Hermann *et al.*, 1992), as follows: the DSDF mixture and the vector were both double-digested with *EcoRI* and *BamHI*, and the DSDF were ligated to the vector DNA. The ligation mixture was transformed into *E. coli* as described in example 1, with selection for ampicillin resistance. Plasmid DNA was isolated from 500 individual clones and screened for the presence of putative promoter fragments by digestion with the restriction enzymes, *EcoRI* and *BamHI*.

17 clones were found to have an *EcoRI* - *BamHI* insert of approximately 200 bp. Plasmid DNA from these 17 clones were transformed into *S. cerevisiae*, with selection for the URA3 marker carried by the plasmids and assayed for production of β -galactosidase. Figure 4 shows the resulting activities of β -galactosidase for the 17 clones. All the promoters have higher activities than the cloning vector itself without promoter fragment inserted (pYLZ-2). More important however, also in this case the clones cover a range of promoter activities in small steps of activity change.

Sequence analysis revealed that the 17 clones discussed above had a perfect TATA box (TATAAA) between the spacer 1 and spacer 2, while two of the 17 clones, YP24 and YP435 each had a defect in the UASGCN4p. However, the activity of YP435 was 39 units which is close to the value obtained with YP212. These data then suggest that the impact of the random sequence of the spacers on the promoter strength, is stronger than the impact of the state of the UASGCN4p binding site.

As discussed above, the artificial yeast promoters had built in two different regulatory features. One is that the promoters should be regulated by the presence of arginine in the growth medium. To test whether the artificial yeast promoters were also regulated by arginine, we grew a number of clones in minimal medium, with and without arginine (SD + 2% glucose; SD + 2% glucose + 1 g/l arginine). Figure 5 shows the result of these experiments. The clones, YP18, YP212, YP435 were indeed regulated 5, 8, 15 fold, respectively, by the presence of arginine. YP183 was not regulated by arginine, and sequence analysis confirmed that this promoter clone had a 13 bp deletion in the arginine repressor binding site.

We also tested whether the promoters were regulated by external amino acids in the growth medium, by analyzing the promoter activity of some of the yeast promoter clones in minimal medium (SD + 2% glucose) and rich medium (SC + 2% glucose-URA). Figure 6 shows the result of these experiments. Indeed, the promoters present in clones YP18, YP212, YP435, and YP183 were regulated from 2 to 10 fold, by the presence of amino acids. YP24 was not regulated, in accordance with the error that had occurred in the UAS_{GCN4p} site on this clone, see above.

The results on amino acid and specific arginine regulation demonstrate that the random spacers method of the invention can be used for generating promoters which cover a broad range of promoter activities and which can be regulated by external signals. The regulatory aspect of the invention is here exemplified by the amino acid and arginine regulation, but is not limited to these cases.

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regulatory analysis of the *Lactococcus lactis* dnaJ gene.
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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Artificial promoter libraries for selected organisms and promoters derived from such libraries

(iii) NUMBER OF SEQUENCES: 58

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: DK 886/96
- (B) FILING DATE: 23-AUG-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 26..82
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Artificial promoter library"
/note= "A degenerated sequence specifying a mixture of artificial promoters covering a wide range of expression in small steps in L. lactis"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 31..45
- (D) OTHER INFORMATION: /standard_name= "Consensus sequence"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature

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      (B) LOCATION:60..69
      (D) OTHER INFORMATION:/standard_name= "Consensus
          sequence"

(ix) FEATURE:
      (A) NAME/KEY: misc_feature
      (B) LOCATION:74..82
      (D) OTHER INFORMATION:/standard_name= "Consensus
          sequence"

(ix) FEATURE:
      (A) NAME/KEY: -35_signal
      (B) LOCATION:40..45
      (D) OTHER INFORMATION:/standard_name= "-35 box"

(ix) FEATURE:
      (A) NAME/KEY: -10_signal
      (B) LOCATION:63..68
      (D) OTHER INFORMATION:/standard_name= "Pribnow box"

(ix) FEATURE:
      (A) NAME/KEY: misc_recomb
      (B) LOCATION:3..25
      (C) IDENTIFICATION METHOD: experimental
      (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
          /standard_name= "Multiple cloning site"
          /label= MCS
          /note= "A sequence specifying recognition sites for the
restriction endonucleases: NlaIV, BstYI, BamHI, AlwI,
MboI, DpnI, AflII, MseI, SspI, NsiI."

(ix) FEATURE:
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      (B) LOCATION:74..98
      (C) IDENTIFICATION METHOD: experimental
      (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
          /standard_name= "Multiple cloning site"
          /label= MCS
          /note= "A sequence specifying recognition sites for the
restriction endonucleases: ScaI, RsaI, HpaI, HincII, MseI,
SfcI, PstI, Fnu4HI, BbvI, PvuII, NspBII, AluI, EcoRI."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
CGGGATCCTT AAGAATATTA TGCATNNNNN AGTTTATTCT TGACANNNNN NNNNNNNNT      60
GGTATAATAN NANAGTACTG TTAAGTGCAG CTGAATTCGG                          100

(2) INFORMATION FOR SEQ ID NO: 2:

  (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 113 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA (genomic)

  (iii) HYPOTHETICAL: YES

  (iv) ANTI-SENSE: NO

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(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:23..95
- (D) OTHER INFORMATION:/standard_name= "Artificial promoter library"
/note= "A degenerated sequence specifying a mixture of artificial temperature regulated promoters covering a wide range of expression in small steps in *L. lactis*"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:23..49
- (D) OTHER INFORMATION:/standard_name= "Sequence providing temperature regulation to promoters"
/note= "This sequence comprising two inverted repeats separated by a short spacer provides temperature (heat shock) regulation to promoters in Gram-positive bacteria"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:50..60
- (D) OTHER INFORMATION:/standard_name= "Consensus sequence"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:75..84
- (D) OTHER INFORMATION:/standard_name= "Consensus sequence"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION:89..95
- (D) OTHER INFORMATION:/standard_name= "Consensus sequence"

(ix) FEATURE:

- (A) NAME/KEY: -35_signal
- (B) LOCATION:55..60
- (D) OTHER INFORMATION:/standard_name= "-35 box"

(ix) FEATURE:

- (A) NAME/KEY: -10_signal
- (B) LOCATION:78..83
- (D) OTHER INFORMATION:/standard_name= "Pribnow box"

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION:3..22
- (D) OTHER INFORMATION:/standard_name= "Multiple cloning site"
/label= MCS
/note= "A sequence specifying recognition sites for the restriction endonucleases: NlaIV, BstYI, BamHI, AlwI, MboI, DpnI, HindIII, AluI, MseI (2 sites), SspI, AseI."

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION:89..111
- (D) OTHER INFORMATION:/standard_name= "Multiple cloning site"
/label= MCS
/note= "A sequence specifying recognition sites for the

restriction endonucleases: ScaI, RsaI, SfcI, PstI, Fnu4HI, BbvI, PvuII, NspBII, AluI, XbaI, MaeI, EcoRI, ApoI."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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CGGGATCCAA GCTTAATATT AATTAGCACT C>NN>NN>NN>NN GAGTGCTAAT TTTTGTGACA      60
NN>NN>NN>NN>NN NNNNTGGTAT AATANNANAG TACTGCAGCT GTCTAGAATT CGG          113
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(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 199 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Saccharomyces cerevisiae*
- (ix) FEATURE:
 - (A) NAME/KEY: protein_bind
 - (B) LOCATION:10..16
 - (D) OTHER INFORMATION:/function= "Activating promoters in *S. cerevisiae*"
 /bound_moiety= "GCN4 protein"
 /standard_name= "Upstream activating sequence"
 /label= UAS GCN4p
 /note= "A DNA sequence that specifies a binding site for the GCN4 protein, which activates the transcription of genes involved in aminoacid synthesis in *S. cerevisiae*."
- (ix) FEATURE:
 - (A) NAME/KEY: TATA_signal
 - (B) LOCATION:67..72
 - (D) OTHER INFORMATION:/standard_name= "TATA box"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_signal
 - (B) LOCATION:122..144
 - (D) OTHER INFORMATION:/function= "Transcription initiation"
 /standard_name= "TI box"
- (ix) FEATURE:
 - (A) NAME/KEY: protein_bind
 - (B) LOCATION:122..144
 - (D) OTHER INFORMATION:/bound_moiety= "Arginine repressor"
 /standard_name= "arginine repressor binding site"
 /label= argR
- (ix) FEATURE:
 - (A) NAME/KEY: misc_RNA
 - (B) LOCATION:145..192
 - (D) OTHER INFORMATION:/function= "Spacer"
 /standard_name= "Part of native sequence for ARG8"

gene incl. first codon"

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION:3..8
- (D) OTHER INFORMATION:/standard_name= "Recognition site
for restriction endonuclease EcoRI"
/label= EcoRI_site

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION:192..197
- (D) OTHER INFORMATION:/standard_name= "Recognition site
for restriction endonuclease BamHI"
/label= BamHI_site

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:10..192
- (D) OTHER INFORMATION:/standard_name= "Artificial
promoter library"
/note= "A degenerated sequence specifying a mixture of
artificial promoters covering a wide range of expression
in small steps in *S. cerevisiae*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAGAAATTCGT GACTCANNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN	60
NNNNNNNNNN NNNNNNTATA AANNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN	120
NCTCTTAAGT GCAAGTGA CT GCGAACATTT TTTTCGTTTG TTAGAATAAT TCAAGAATCG	180
CTACCAATCA TGGATCCCCG	199

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Pseudomonas putida*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:1..45
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Artificial promoter library"
/note= "A degenerated sequence specifying a mixture of
artificial promoters covering a wide range of expression
in small steps in *Pseudomonas putida*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

NNNNNNNNNTT GRNNNNNNNN NNNNNNNNNN NTATRATNNN NNNNN

45

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CATACCGGAG TTTATTCTTG ACAGTTCCAC CTCGGGTGA TATAATATCT CAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional promoter"
/label= Cp10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CATGGCTTAG TTTATTCTTG ACAGGGTAGT ATCACTGTGA TATAATAGGA CAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..59
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CATAAGTGAG TTTATTCTTG ACCCGGACGC CCCCTTTGA TATAATAAGT AGTACTGTT

59

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CATATACAAG TTTATTCTTG AACTAGTCG GCCAAAATGA TATAATACCT GAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional
promoter"
/label= Cp13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CATGCTTTAC TTTATTCTTG ACAAACCAC CAGCTTTTGG TATAATACGT GAGAACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional
promoter"
/label= Cp14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CATGACGGAG TTTATTCTTG ACACAGGTAT GGACTTATGA TATAATAAAA CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CATTACNTAG TTNATTCTTG ACAGAATTAC GATTCGCTGG TATAATATAT CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..58

(D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp16

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CATTGTGTAG TTTATTCTTG ACAGCTATGA GTCAATTTGG TATAATAACA GTACTCAG 58

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..59

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CATTCTGGAG TTTATTCTTG ACCGCTCAGT ATGCAGTGGT ATAATAGTAC AGTACTGTT

59

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..58

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATTTTGCAG TTTATTCTTG ACATTGTGTG CTCGGGTGT ATAATACTAA GTACTGTT

58

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..58

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CATCGCTTAG TTTTCTTGA CAGGAGGGAT CCGGGTTGAT ATAATAGTTA GTACTGTT

58

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CATTTGCTAG TTTATTCTTG ACATGAAGCG TGCCTAATGG TATATTACTT GAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:4..60
- (D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CATGGGTGAG TTTATTCTTG ACAGTGC GGC CNGGGGCTGA TATCATAGCA GAGTACTATT 60

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..59
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CATTACCGAG TTTATTCTTG ACACCGTTTA TCGGGGTTGT ATAATACTAT AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CATGTAGGAG TTTATTCTTG ACAGATTAGT TAGGGGGTGG TATAATATCT CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: YES

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Lactococcus lactis*

- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION: 4..60
 - (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp24

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CATGGGTAAG TTTATTCTTC AACTATCTG GCGCGATGG TATAATAAGT GACTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 59 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: YES

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Lactococcus lactis*

- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION: 3..59
 - (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp25

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTTTGGCAGT TTATTCTTGA CATGTAGTGA GGGGGCTGGT ATAATCACAT AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional
promoter"
/label= Cp26

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CATTCTACAG TTTATTCTTG ACATTGCACT GTCCCCCTGG TATAATAACT ATACATGCAT 60

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional
promoter"
/label= Cp28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CATGGGGCCG TTTATTCTTG ACAACGGCGA GCAGACCTGG TATAATAATA TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Lactococcus lactis*
- (ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 4..59
(D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp29
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CATCGGTAAG TTATTCTTGA CATCTCAGGG GGGACGTGGT ATAATAACTG AGTACTGTT

59

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Lactococcus lactis*
- (ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 4..60
(D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp3
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CATCCTGTAG TTTATTCTTG ACACACGTNN TTAGCTGTGG TATAATAGGA GAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CATGACAGAG TTTATTCTTG ACAGTATTGG GTTACTTTGG TATAATAGTT GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CATACGGGAG TTTATTCTTG ACATATTGCC GGTGTGTTGG TATAATAACT TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CATGTTGGAG TTTATTCTTG ACATACAATT ACTGCAGTGA TATAATAGGT GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION:4..60
(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp34

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CATCGCGAAG TTTATTCTTC ACACACCGCA GAACTTGTGG TATAATACAA CAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactococcus lactis

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION:4..59
(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp37

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CATCATTAAG TTTATTCTTC ACATTGGCCG GAATTGTTGT ATAATACCTT AGTACTGTT

59

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CATAGAGAAG TTTATTCTTG ACAGCTAACT TGGCCTTTGA TATAATACAT GAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 4..60
- (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CATTGCGAAG TTTATTCTTG ACAGTACGTT TTTACCATGA TATAATAGTA TAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Lactococcus lactis*
- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION: 4..60
 - (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp4
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GATGTTTTAG TTTATTCTTG ACACCGTATC GTGCGCGTGA TATAATCGGG ATCCTTAAGA 60

(2) INFORMATION FOR SEQ ID NO: 34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 59 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Lactococcus lactis*
- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION: 4
 - (D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp40
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CATAGAACAG TTTATTCTTG ACATTGAATA AGAAGGCTGA TATAATAGCC AGTACTGTT 59

(2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional
promoter"
/label= Cp41

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CATCCGCAAG TTTATTCTTG ACAGCTGAAT GTAGACGTGG TATAATAGTT AAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..60

(D) OTHER INFORMATION: /standard_name= "Constitutional
promoter"
/label= Cp42

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CATTCGTAAG TTTATTCTTG ACACCTGAGA TGAGGCGTGA TATAATAAAT AAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..59

(D) OTHER INFORMATION: /standard_name= "Constitutional promoter"
/label= Cp44

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CATCGGGTAG TTTATTCTTG ACAATTAAGT AGAGCCTGAT ATAATAGTTC AGTACTGTT

59

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 4..59

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Constitutional promoter"
/label= Cp5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CATGGGGGAG TTTATTCTTG ACATCATCTT CGTAGCCTGG TATACTACAT GAGTATGTT

59

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CATGTGGGAG TTTATTCTTG ACACAGATAT TTCCGGATGA TATAATAACT GAGTACTGTT

60

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60

(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

TATGCGGTAG TTTATTCTTG ACATGACGAG ACAGGTGTGG TATAATGGGT CTAGATTAGG

60

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:4..60
(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CATTCTTTAG TTTATTCTTG ACAAACGTAT TGAGGACTGA TATAATAGGT GAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Lactococcus lactis*

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION:4..60
(D) OTHER INFORMATION:/standard_name= "Constitutional
promoter"
/label= Cp9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CATAGTCTAG TTTATTCTTG ACACGCGGTC CATTGGCTGG TATAATAATT TAGTACTGTT 60

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION:8..177
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GAATTCGTGA CTCAAACGGG TGGTCGACGG GTGGTTCCAA TTAATTGGCG TCCCTCTTAT 60
AAAGGCGAGG GTACGTGCGA CAATTGGTAG AGCGAGCGGG GCTCTTAAGT GCAAGTGACT 120
GCGAACATTT TTTTCGTTTG TTAGAATAAT TCAAGAATCG CTACCAATCA TGGATCC 177

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..181
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Ypl12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GAATTCGTGA CTCACGGCAT CTGATGGTTG ACCATAGTCA GGAACATTGT GCTGGAGTTC 60
CTTGAGGAAT GAGTTATAAA ATGGGAGGTT GCGGCTAATG CCAGGCAGGA GAGGAACCCT 120
CTTAAGTGCA AGTGACTGCA AACATTTTTT TCGTTTGTG AATCGCTACC AATCATGGAT 180
CC 182

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:8..181
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```
GAATTCGTGA CTCACTAGGC AGGTCACGTT GGCTCTTCGC GGCGCAGGTT CGTATGCCGC      60
GCCGCCAGGG GCTTTATAAA GGTCGTCCTG GGTACAGTTG GGATGGCTCC ACGTTTCGGC      120
TCTTAAGTGC AAGTGACTGC GAACATTTTCG TTTGTTAGAA TAATTCAAGA ATCGCTACCA      180
ATCATGGATC C                                                                191
```

(2) INFORMATION FOR SEQ ID NO: 46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 167 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Saccharomyces cerevisiae*
- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION:8..167
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

```
GAATTCGTGA CTCAGGGCCG TACTAAGTAG CTTTCGTATG CTATGCGGGG TTTTATAAAT      60
CTTTGGGCCA TGGTCTTGCT GGAAACACC TCTCTTAAGT GCAAGTGACT GCGAACATTT      120
TTTTCGTTTG TTAGAATAAT TCAAGAATCG CTACCAATCA TGGATCC                      167
```

(2) INFORMATION FOR SEQ ID NO: 47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 191 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:8..191

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION:/evidence= EXPERIMENTAL

/standard_name= "Yeast promoter"

/label= Yp154

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

GAATTCGTGA CTCACCGCTC GGGTGCAGGG CCAAGGCGGC GGAATGTGCG GGGCGTTCTA	60
GCGCAATCGG GGTATAAATT TATAAGGAGG CTGCGGGTGC TAGTTTGTCT AGTTTGACTC	120
TTAAGTGCAA GTGACTGCGA ACATTTTTTCG TTTGTTAGAA TAATTCAAGA ATCGCTACCA	180
ATCATGGATC C	191

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 195 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION:8..190

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION:/evidence= EXPERIMENTAL

/standard_name= "Yeast promoter"

/label= Yp18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GAATTCGTGA CTCAGGATTA GCTATGCCGG TTGGGATAAG CGAACAACTG GAGGTGAGAA	60
GCTTTTTTGTG AGAATATAAA CCCGTTAGTC AGGGTTTGGT GGGATAGGGG GTACTGTACC	120
TCTTAAGTGC AAGTGACTGC GAACATTTTTT TTCGTTTGTT AGAATAATTC AAGAATCGCT	180
ACCAATCATG GATCC	195

(2) INFORMATION FOR SEQ ID NO: 49:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 179 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Saccharomyces cerevisiae*
- (ix) FEATURE:
 (A) NAME/KEY: promoter
 (B) LOCATION: 8..179
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
 /standard_name= "Yeast promoter"
 /label= Yp183
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

GAATTCGTGA CTCACTAAGG GTTCGCCATT AACAGAATCG CTGGTAGAAC ATCGGTAGTT	60
AGGCACCCGA GTATAAACAG GCGGACCCCT CACGGATATC AGCTGATAGT GCGAGCCTCA	120
ATGCGAACAT TTTTTCGTT TGTTAGAATA ATTCAAGAAT CGCTACCAAT CATGGATCC	179

(2) INFORMATION FOR SEQ ID NO: 50:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 195 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Saccharomyces cerevisiae*
- (ix) FEATURE:
 (A) NAME/KEY: promoter
 (B) LOCATION: 8..190
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
 /standard_name= "Yest promoter"
 /label= Yp190
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GAATTCGTGA CTCAGTATCC ACGGGTGTTT GAGGGCTGGT CGCAGGTTAG CAGGCGAGGG	60
CGGGTGGTTA CGGCTATAAA TGAGTGTTTG CAGCCGGGTA CGGGCGTACG AGTAGTGATC	120

TCTTAAATGC AAGTGACTGC GAACATTTTT TTCGTTTGGT AGAATAATTC AAGAATCGCT 180
ACCAATCATG GATCC 195

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 193 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..189
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yest promoter"
/label= Yp191

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

GAATTCGTGA CTCAATGCTG CGGGCGGCAG GAGTCTGGTG TAACTCCCA TTTTGAGTGA 60
AAGACAGACC ATCTATAAAC ATTTGGTGGG CAAAGTGGCC TGGCGGATTG GTTTGGACTC 120
TTAAGTGAAG GTGACTGCGA ACATTTTTTTT CGTTTGTTAG AATAATTCAA GAATCGCTAC 180
CAATCATGGA TCC 193

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 166 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..166
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL

/standard_name= "Yeast promoter"
/label= Yp192

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GAATTCGTGA CTCACTTAAG GCTACTGCGG AAGTTTAGAT CTAAGGTCGG AAATAATTTA 60
GAAAAATTACG ACATTATAAA TAGCGGAGAG GCCAGGTGAT GGGCACCATT GTGGGGGGGC 120
TCTTAATTGT TAGAATAATT CAAGAATCGC TACCAATCAT GGATCC 166

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..190
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp212

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GAATTCGTGA CTCAGTCGCC CGCAAGATGG GATGGTGCAT TTAAACACC CGAATTATAC 60
TCGTCAACTT ATAGTATAAA CGGAACGCGA CGATACGTTT TAGTTTTTCGG CGAAGTCGAC 120
TCTTAAGTGC AAGTGACTGC GAACATTTTT TTCGTTTGT AGAATAATTC AAGAATCGCT 180
ACCAATCATG GATCC 195

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:8..183
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

```
GAATTCGTAC TCACGACAGC GTTATGACTT CGAGGACCAG CTA CTTCCGG TCGCGTACTA      60
GTTTTTACCT GTATAAACTT TGCTACCGCT GGGCCTTGGT GGTGCTGTCC CGCTCTTAAG      120
TGCAAGTGAC TGCGAACATT TTTTTCGTTT GTTACAATAA TTCAAGAATC GCTACCAATC      180
ATGGATCC                                         188
```

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION:8..190
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION:/evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp435

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

```
GAATTCGTGA CTAAATGGAT AAGGTTATCG CCATCACGGA GTCTTCTCTC ACGTCTGGAG      60
CAGAGGCTAG ACCTTATAAA TTATACATGG TGGGAGAGGC GATAGTCTTT AGAGACGTGC      120
TCTTAAGTGC AAGTGACTGC GAACATTTTTT TTCGTTTGTGTT AGAATAATTC AAGAATCGCT      180
ACCAATCATG GATCC                                         195
```

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 189 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 8..184

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp68

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GAATTCGTGA CTCACAAGAA TGTGGGCGGG TCGTTAACT GAGCCTGGAC ACCTTGGCTG	60
CGTCGCTTTC GTATAAAGAT CTTAGAGCTG TGGAGTCTGG GTCGAGTGGC CAGCTCTTAA	120
ATGCAAGTGA CTGCGAACAT TTTTTCGTT TGTTAGAATA ATTCAAGAAT CGCTACCAAT	180
CATGGATCC	189

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 195 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

(A) NAME/KEY: promoter

(B) LOCATION: 8..190

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp89

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GAATTCGTGA CTCACTCGGA AGATTGGGTT TACGATTAGG ATGGCGCGGC AGAACCGGGG	60
GGGATTCCCT TCTATATAAA GGGTTCCGAT ACTACGTGCT GCGGACGGCC GATCGAGTTA	120
TCTTAAGTGC AAGTGACTGC GAAAATTTT TTCGTTTGT AGAATAATTC AAGAATCGCT	180

ACCAATCATG GATCC

195

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 176 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(ix) FEATURE:

- (A) NAME/KEY: promoter
- (B) LOCATION: 8..171
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /evidence= EXPERIMENTAL
/standard_name= "Yeast promoter"
/label= Yp96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GAATTCGTGA CTCATCTAGT GAGAGGAGCC GTGGTATCTT GTGTCACCAC CAGGGGAAAA	60
TAATGGCAGG GGTGTATAAA TGGTCGAGTA GTCGCGACCC ACGCTGCAAG GCAAGGAACT	120
CTTAAATTTT TTCGTTTGT TAGAATAATT CAAGAATCGC TACCAATCAT GGATCC	176

International patent application No. PCT/DK97/00342

Peter Rudahl Jensen

Artificial promoter libraries for selected organisms and
promoters derived from such libraries

5 Our ref: 20911 PC 1

NEW CLAIMS, 26 NOVEMBER 1998

- 10 1. A promoter library suitable for optimising the expression of a gene in a selected
organism or group of organisms, said library comprising a set of different individual
promoter sequences covering, with respect to promoter strength for said gene, a
range of promoter activities, the set of different individual promoter sequences
comprising double stranded DNA sequences, the sense strands of which comprise
15
at least two consensus sequences of a promoter sequence identified in said organism
or group of organisms, at least half of each of said consensus sequences is kept
constant in all of the individual promoter sequences and, between said consensus
sequences or flanking at least one of said consensus sequences, a nucleotide spacer
20 sequence, at least part of which, relative to the corresponding spacer sequence of the
identified promoter, is varied to comprise nucleotides that are selected randomly
among the nucleobases A, T, C and G,
- the promoter library spanning, with respect to promoter activities for said gene, a
25 range of interest, in small steps, each step preferably changing the activity by 50-
100%.
2. A promoter library according to claim 1 wherein at least 10 nucleotides in the
spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.
- 30 3. A promoter library according to claim 1 wherein the promoter sequences comprise a
regulatory DNA sequence imparting a specific regulatory feature to the promoters of
said promoter sequences.

AMENDED SHEET

4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease
5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.
6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -10 signal TATAAT.
- 10 7. A promoter library according to claim 5 or 6 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -35 signal TTGACA.
8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.
- 15 9. A promoter library according to any of claims 5-8 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42
10. A promoter library according to claim 7 wherein the spacer sequence between the -35 and the -10 signal is 14-23 bp.
- 20 11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.
- 25 12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.
13. A promoter library according to claim 12 wherein the consensus sequences
- 30 comprise a TATA box and at least one upstream activation sequence (UAS).
14. A promoter library according to claim 12 or 13 wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

AMENDED SHEET

15. A promoter library according to any of claims 12-14 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

5 16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising
10 at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-
15 conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences constant, and

20 (iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

25

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method
30 comprising

(i) selecting from the promoter library of any of claims 1-15 a set of promoters covering a desired range of promoter activities,

APPROVED FOR

(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene
5 product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

10

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and a eukaryotic organism.

21. A method of isolating a promoter sequence being capable of optimizing the
15 expression of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16 or 17, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

20 (ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

25

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation.

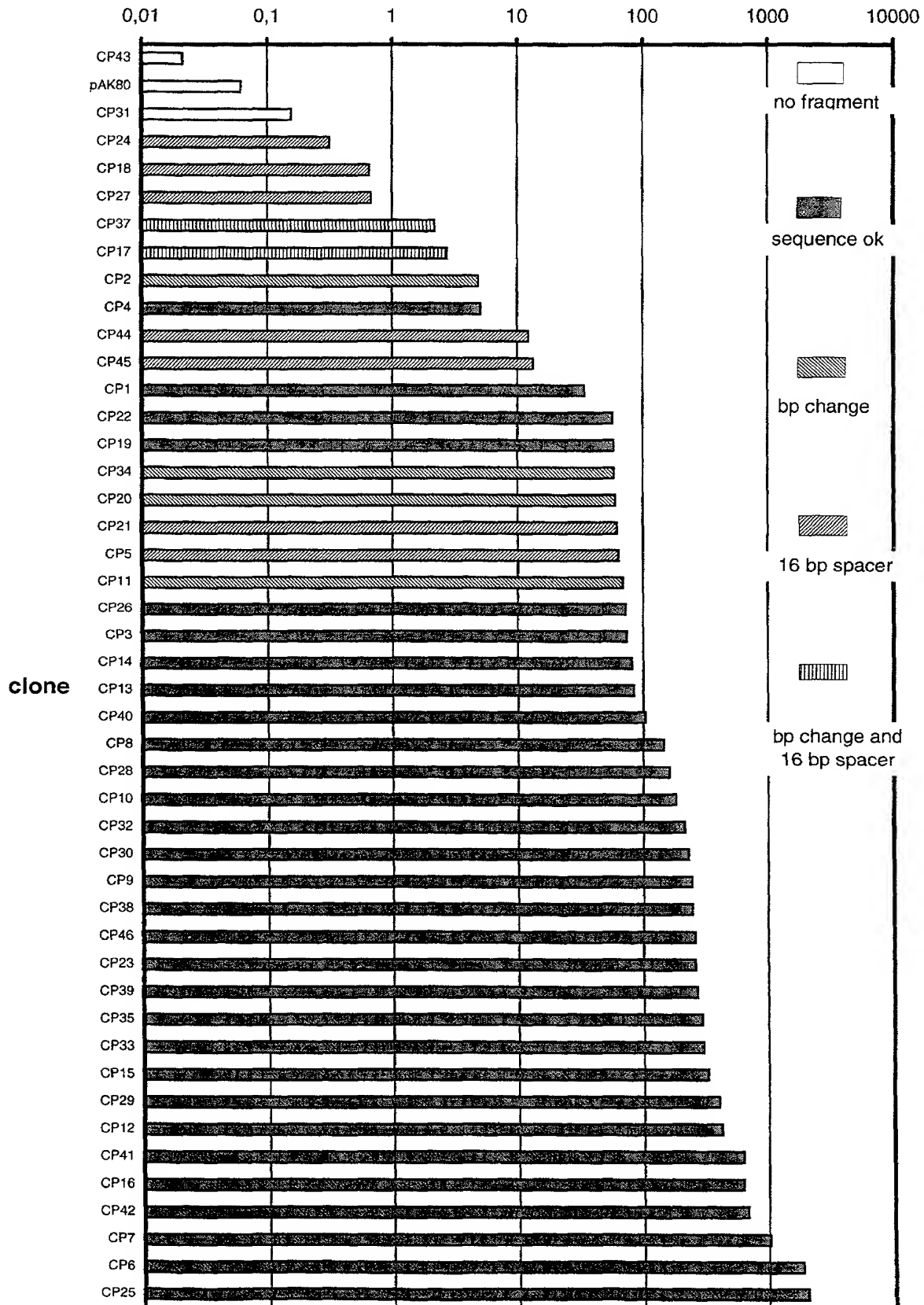
22. A promoter sequence that is capable of optimising the expression of a gene in a
30 selected organism, the promoter sequence is obtainable by the method of claim 21.

ACCEPTED

1/5

Fig. 1.

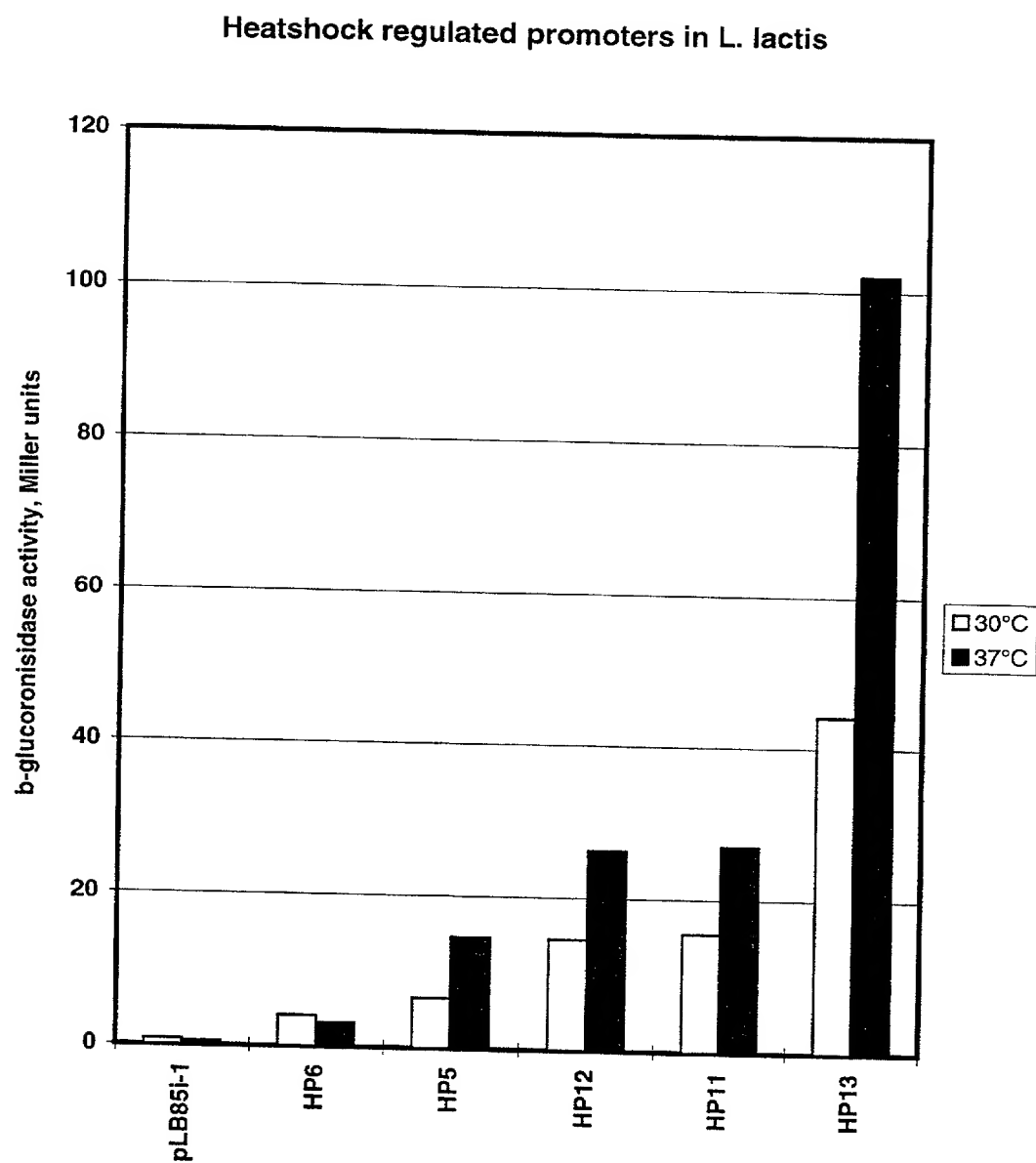
activity (Miller units)



SUBSTITUTE SHEET

2/5

FIG. 2



3/5

Fig. 3.

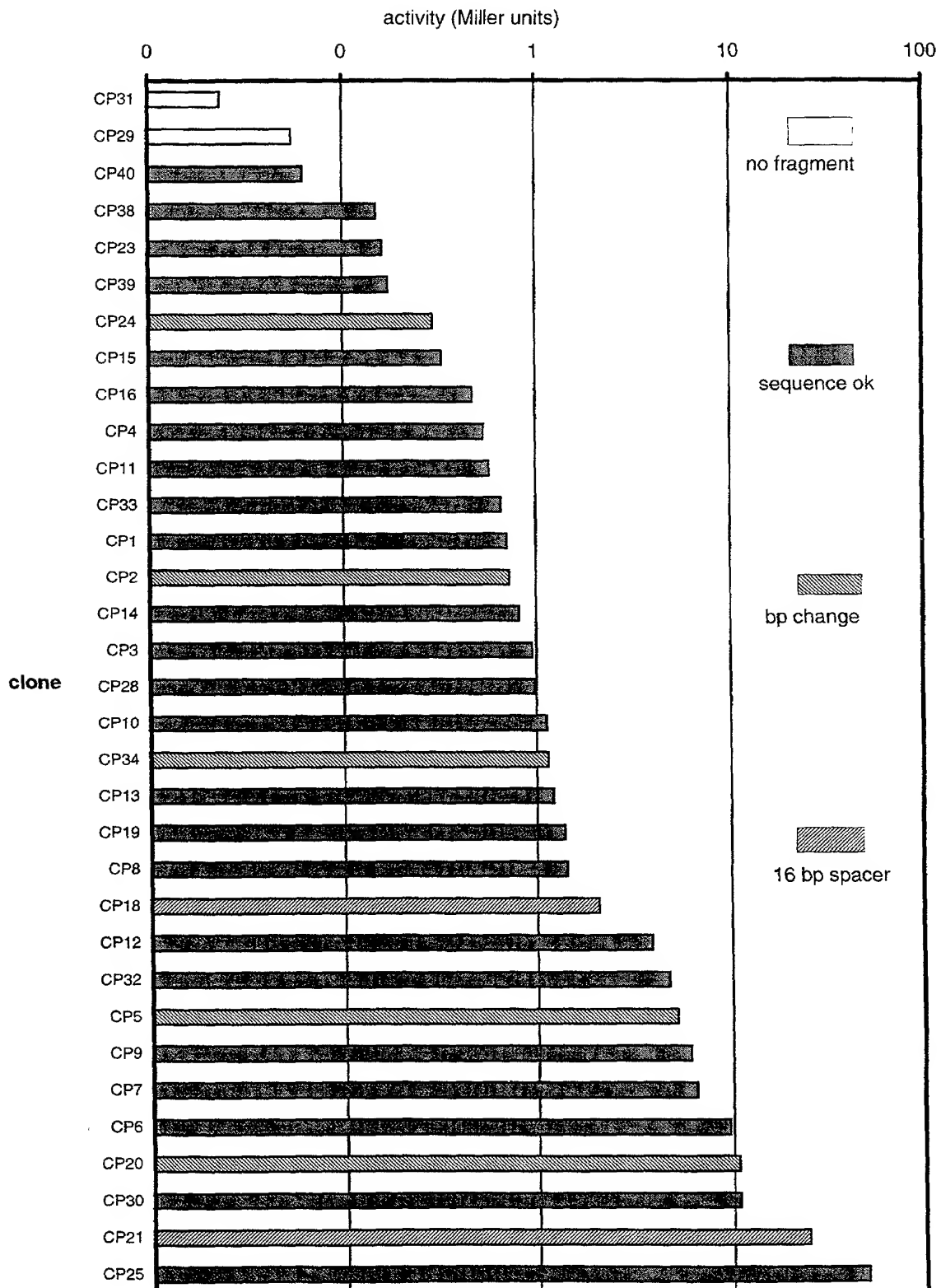
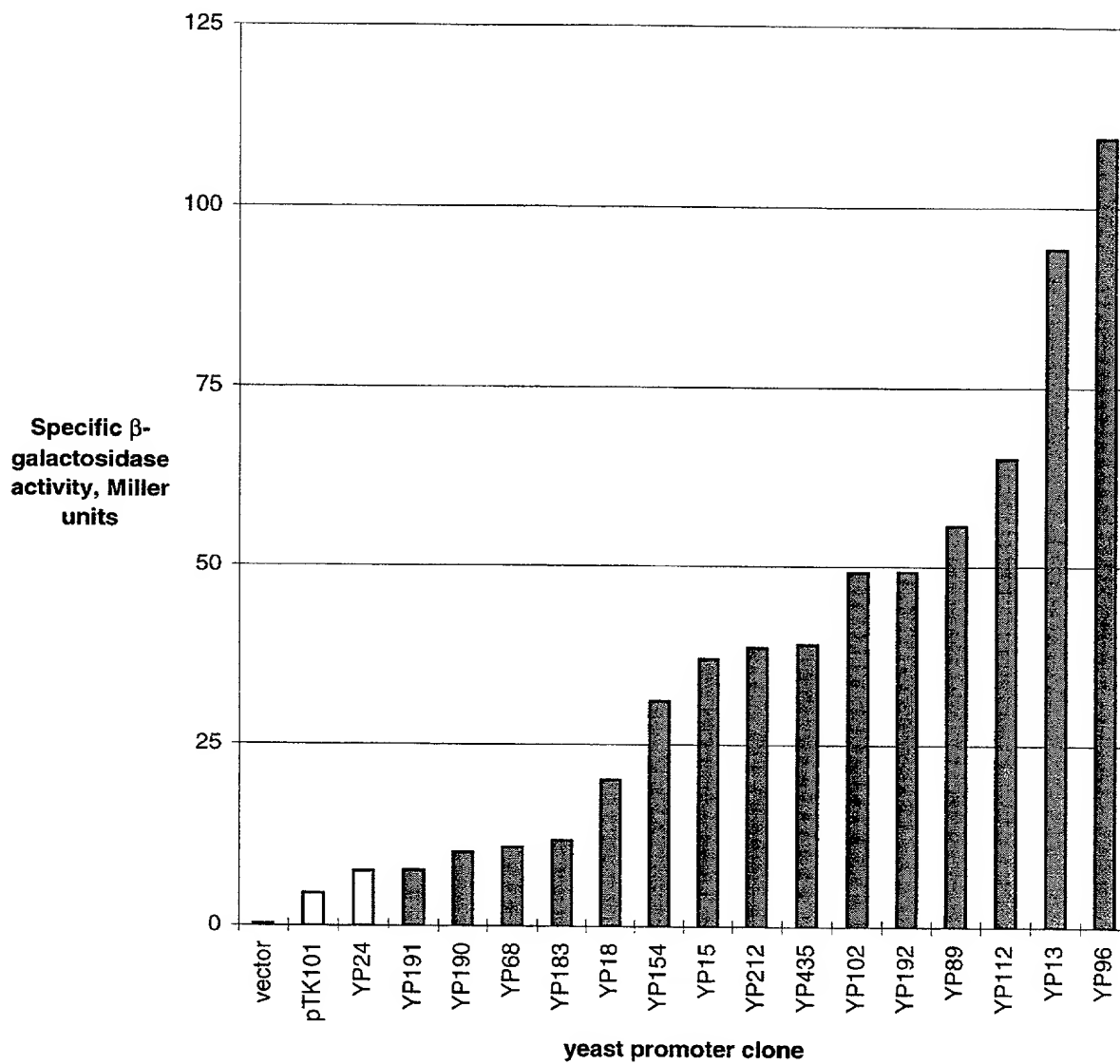


Fig. 4

Artificial yeast promoters



5/5

FIG. 5

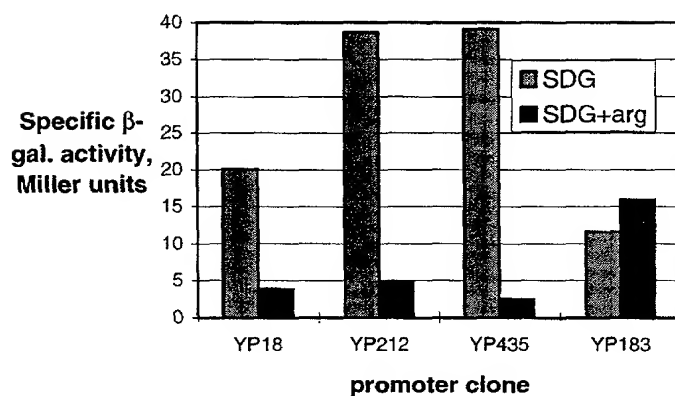
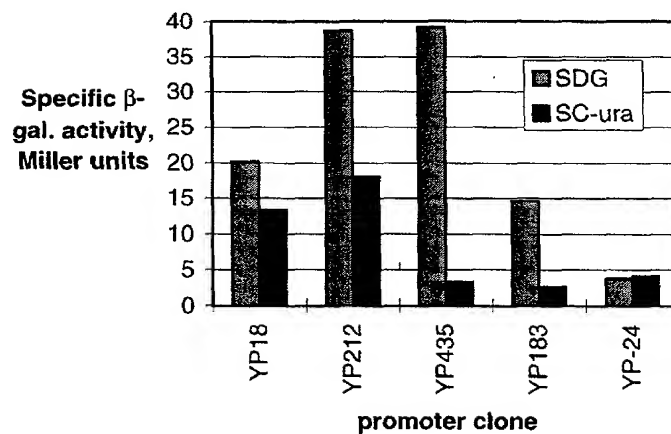
Artificial yeast promoters, regulation by external arginine

FIG. 6

Artificial yeast promoters, general regulation by external amino acids

JOINT DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenship are as stated below next to our names;

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled ARTIFICIAL PROMOTER LIBRARIES FOR SELECTED ORGANISMS AND PROMOTERS DERIVED FROM SUCH LIBRARIES, the specification of which

(X) is attached hereto.

() was filed on _____ as Application Serial Number _____ and was amended on _____ (if applicable)

(X) Is an International Application, PCT Application No. PCT/DK97/00342 filed on August 25, 1997.

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to in this declaration.

We acknowledge the duty to disclose all information known to us to be material to the patentability of this application, as defined in 37 C.F.R. § 1.56.

We acknowledge the duty to disclose to the Office all information known to us to be material to patentability as defined in § 1.56, which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

Prior Foreign Application(s)

We hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Country	Application Number	Date of Filing (day, month, year)	Date of Issue (day, month, year)	Priority Claimed Under 35 U.S.C. 119
DENMARK	DK 0886/96	23 August 1996		Yes [X] No []
				Yes [] No []

Prior United States Provisional Application(s)

I hereby claim the benefit under 37 C.F.R. § 119(e) of any United States provisional application(s) listed below

Application Number	Filing Date

Prior United States Application(s)

We hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial Number	Date of Filing (day, month, year)	Status - Patented, Pending, Abandoned

And we hereby appoint, both jointly and severally, as our attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith the following attorneys or agents, their registration numbers being listed after their names:

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We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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